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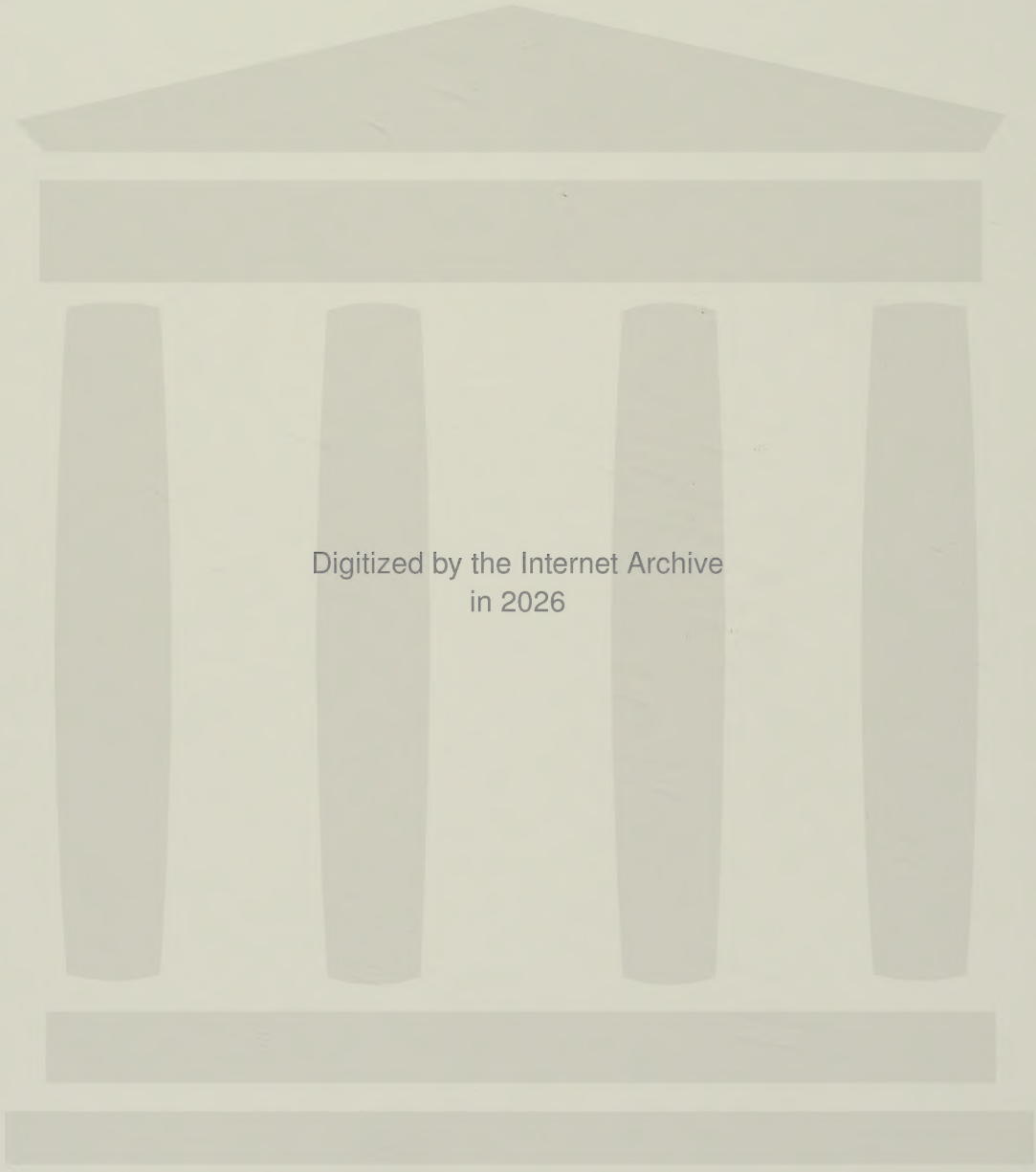

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Preface

The 37th Joint Meeting of the UJNR Aquaculture Panel was conducted from October 27th – November 1st at Yokohama (Kanagawa), Tsuchiura, Kamisu (Ibaraki) and Choshi (Chiba). Through the long history of UJNR, Aquaculture Panel has contributed to development of aquaculture researches of both countries by means of various cooperative activities, i.e. the exchange of scientists, the exchange of literatures and promotion of joint research projects, and has given the achievements to aquaculture industry. And, we have been editing "A History of the US/Japan Natural Resource Aquaculture Committee" as the forty years document for taking over the fruits. The present special issue of Bulletin of Fisheries Research Agency is the proceedings of the 37th UJNR Aquaculture Panel Symposium "Aquafeed and Its Future Aspect" that is held in accordance with the 7th UJNR three-year plan. We had a new effort at this symposium. As replacing the past activity of the exchange of literatures, annotated bibliography of key works, which focused on the topic of the symposium, was listed at each abstract of the presentations. The bibliography helps everyone who is interested in the field understand the details of the presentations. At this issue, the annotated bibliography of key works on the topic of the symposium is published as well.

Further development of aquaculture is highly desired as a production source of important foods for human being all over the world. Stable supplies of safe and economical feed ingredients are requisite for the sustainable development of aquaculture. Nevertheless, in recent years we recognized some serious problems, which threatened the use of important feed ingredients like fish meal and oil. On this symposium we UJNR Aquaculture Panel members discussed the problems concerning aquafeeds, and attempted to explore the effective means to overcome these constraints for the further sustainable advancement of aquaculture. It is my great pleasure that the present UJNR proceedings containing high quality papers of the selected American and Japanese aquaculture scientists will help readers understand the problems and future aspects of aquafeed.

Finally I would like to express my sincere gratitude to the colleagues involved in the UJNR Aquaculture Panel for their efforts to prepare and organize the symposium. I also would like to deeply thank the editorial board members for publishing the proceedings.

Takaji Iida
Chair of UJNR Aquaculture Panel
Director General
National Research Institute of
Aquaculture
Fisheries Research Agency

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Program

The 37th Scientific Symposium of UJNR Aquaculture Panel

Aquafeed and its future aspects

Yokohama City Port Opening Memorial Hall, Kanagawa
October 28th – 29th, 2008

Aim of the symposium

Further development of aquaculture is highly desired as a production source of important foods for human being all over the world. Stable supplies of safe and economical feed ingredients are requisite for the sustainable development of aquaculture. Nevertheless, in recent years we recognized some serious problems that threatening the use of important feed ingredients like fish meal and oil have newly appeared as our mutual areas of future research.

On this symposium we UJNR aquaculture panel members discuss about the problems deal with aquafeeds and attempt to explore the effective means to overcome these constrains for the further sustainable advancement of aquaculture.

Tuesday, October 28th, 2008 Registration 9:00 - 9:30

Opening remarks (Hiroshi Nakano, Japan Panel Chair) ····· 9:30 - 9:35
Aim of the symposium (Makoto Yamasaki) ····· 9:35 - 9:40

Session I. Current status surrounding aquafeeds and feed researches

(Moderators: Michael Rust and Heisuke Nakagawa)

1. Introduction: Present status of aquafeed researches in Japan ····· 9:40 - 10:00
   Takao Yoshimatsu (Japan)
2. The trend of aquafeed and measures in Japan ····· 10:00 - 10:25
   Masahide Fujise (Japan)
3. Challenges for development of aquafeeds ····· 10:25 - 10:50
   Makoto Nakada (Japan)

Break ····· 10:50 - 11:05

4. Maintaining human health benefits in farmed fish:
   A focused review of the seafood and human health literature ····· 11:05 - 11:30
   Walton Dickhoff (U.S.A.)
5. NOAA/USDA Future of feeds initiative ····· 11:30 - 11:55
   Robert Iwamoto (U.S.A.)

Announcement ····· 11:55 - 12:00
Lunch ····· 12:00 - 13:00

Session Ⅱ : Novel findings for the development of aquafeed researches

(Moderators: Robert Iwamoto and Shuichi Satoh)

6. Over view of the plant feeds in aquaculture working group ····· 13 : 00 - 13 : 25
   Michael Rust (U.S.A.)
7. An overview of progress toward developing an all plant-based diet for rainbow trout ····· 13 : 25 - 13 : 50
   Gibson Gaylord (U.S.A.)
8. Dietary supplementation strategies to improve performance of rainbow trout *Oncorhynchus mykiss* fed plant-based diets ····· 13 : 50 - 14 : 15
   Wendy Sealey (U.S.A.)
9. Taurine : an essential nutrient in fish ····· 14 : 15 - 14 : 40
   Shin-Kwon Kim (Japan)

Break ····· 14 : 40 - 14 : 55

(Moderators: Walton Dickhoff and Takao Yoshimatsu)

10. Development of a new dietary material from unutilized algal resources using fermentation skills ····· 14 : 55 - 15 : 20
    Motoharu Uchida (Japan)
11. Approach to expand Japanese anchovy use as feed material -Efficiency of thiamine supplementation- ····· 15 : 20 - 15 : 45
    Noriko Ishida (Japan)
12. Larval feeds research in the United States ····· 15 : 45 - 16 : 10
    Michael Rust (U.S.A.)
13. Aquafeed and seafood quality ····· 16 : 10 - 16 : 35
    Cheng-Sheng Lee (U.S.A.)
14. Quality control of cultured fish by feed supplements ····· 16 : 35 - 17 : 00
    Heisuke Nakagawa (Japan)

Announcement ····· 17 : 00 - 17 : 05

Wednesday, October 29th, 2008 Registration 9 : 15 - 9 : 30

Session Ⅲ : Problems and new study approaches for breakthrough

(Moderators: Cheng-Sheng Lee and Makoto Yamasaki)

15. Protein production advantages in the face of increasing feed costs: Identifying opportunities within the aquaculture industry ····· 9 : 30 - 9 : 55
    Gina Shamshak (U.S.A.)
16. Availability of gene-modified feed ingredient to fish ····· 9 : 55 - 10 : 20
    Shuichi Satoh (Japan)
17. Quantification of waste feed and fish feces using stable carbon and nitrogen isotopes ····· 10 : 20 - 10 : 45
    Hisashi Yokoyama (Japan)
18. Measurement of swimming behavior and digestive process of cultured fish ····· 10 : 45 - 11 : 10
    Toshinori Takashi (Japan)

Break ····· 11 : 10 - 11 : 25

Session Ⅳ : General discussion ····· 11 : 25 - 11 : 55
(Moderators: Panel members)

Closing remarks (Robert Iwamoto, U.S.A. Panel Chair) ····· 11 : 55 - 12 : 00

水研センター研報，第31号，1－7，平成22年
Bull. Fish. Res. Agen. No. 31, 1-7, 2010

The Issues Surrounding Aquaculture Feeds and the Current Measures Associated with Them in Japan

Masahide FUJISE[*1] and Tomoaki KANMURI[*2]

Abstract : In recent years, the annual aquaculture production of marine fish in Japan has remained stable at about 250,000 tons and has occupied an important position in Japanese marine fish production. However, the high price of fishmeal since 2006 has inflated the price of aquaculture feeds, which has led to the deterioration of management by fish farmers. Therefore, it is necessary to promote sustainable aquaculture production, for example, through development of aquaculture feeds that contain little or no fishmeal and through the introduction of self-feeding systems to prevent excessive use of aquaculture feeds.

Key word : aquaculture feeds, fishmeal, sustainable production

Annual production from marine fish aquaculture in Japan has reached around two hundred fifty thousand tons during the past years (Fig. 1). Highest production has been through the production of yellowtail, mainly *Seriola quinqueradiate* and *S. dumerili*, followed by red sea bream (*Pagrus major*). Those species account for about ninety percent of the production. Other cultured species include coho salmon, olive flounder and globefish.

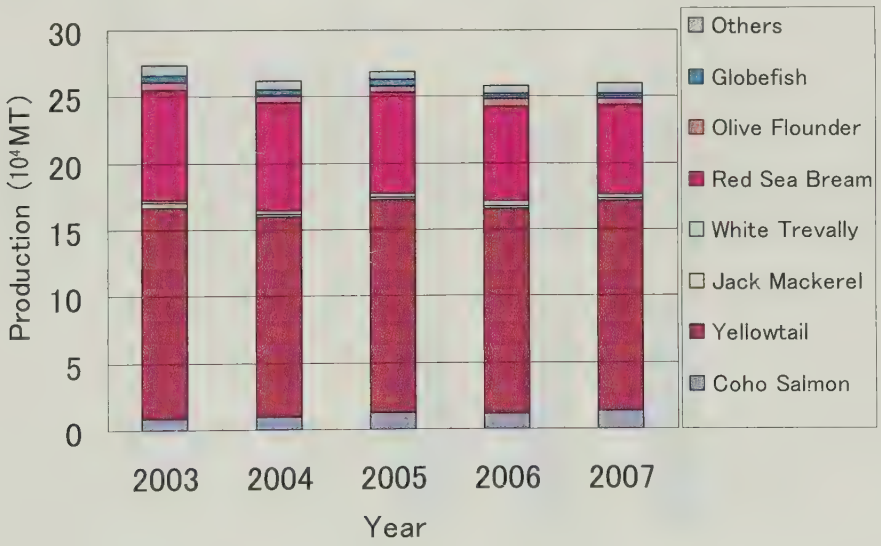

Fig. 1. Recent production of marine fish aquacultured in Japan.
Source : *2003-2007, Annual Statistics on Fishery and Aquaculture Production,* Statistics Department, Ministry of Agriculture, Forestry and Fisheries.

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Mixed Feed

The annual feed amounts used for yellowtail production consist of raw feed used directly and mixed feed which is made in feed processing plants, range from 800,000 to 1,000,000 tons and feed used in conjunction with red sea bream production ranges from 300,000 to 400,000 tons (Fig. 2). Mixed feed accounts for about 40% of yellowtail and 80% of red sea bream production.

Exact annual amounts of feed used is not known, however judging from the data on cultured fish production, feed production of major makers and other information concerning the raw feed marketing trade, we estimate that the production of mixed feed has reached approximately 450,000 tons and the production of raw feed is about 800,000 tons. The details on ingredients for mixed feed production in the last three years in Japan showed fishmeal accounted for more than half (Fig. 3).

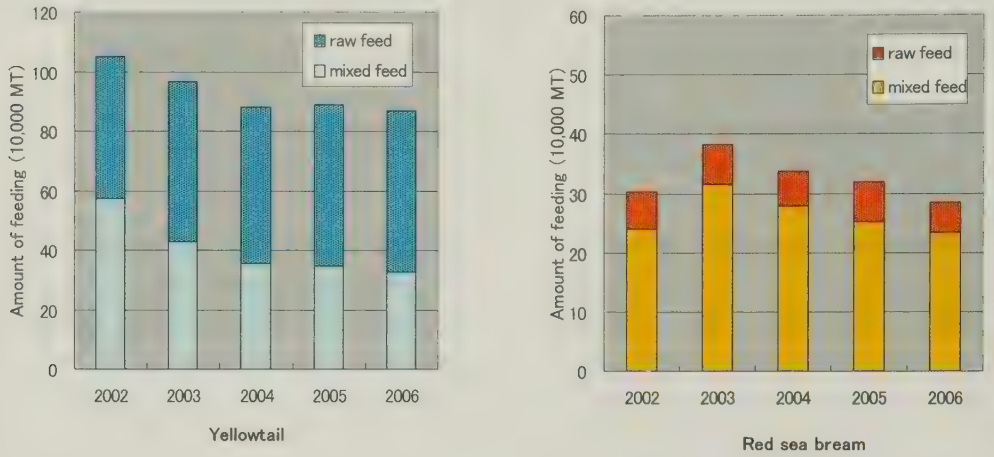


Fig. 2. Amounts of raw feed and mixed feed fed to yellowtail and red sea bream.
Source : *2002-2006, Annual Statistics on Fishery and Aquaculture* Production, Statistics Department, Ministry of Agriculture, Forestry and Fisheries.

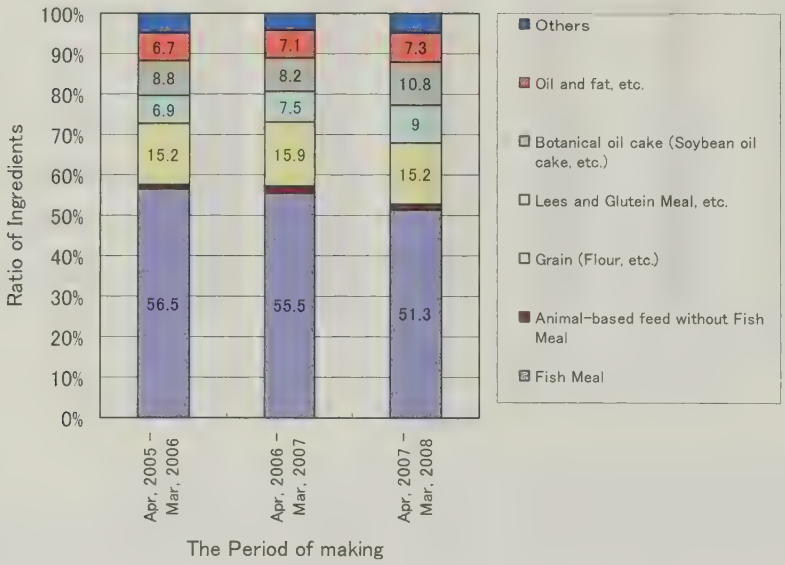


Fig. 3. Raw materials for aquaculture feed in Japan.
Source : *2005-2007, Monthly Feed Statistics, Agricultural Production Bureau,* Ministry of Agriculture, Forestry and Fisheries.

Most of fishmeal is imported from overseas. Peru and Chile are major sources of fishmeal for Japan. Increasing prices of fishmeal, fish oil and other feed ingredients will certainly affect the amount and cost of mixed feeds. The monthly average amount and price of imported fishmeal since January 2004 are shown in Fig. 4. The price has been increasing gradually since 2004, and jumped to ¥140,000 per ton in the middle of 2006.

We don't have any official statistics on the price of mixed feed used for fish aquaculture, either. We were able to obtain information from feed manufacturers and assumed that the price of mixed feed increased by 20 to 30% in 2006 and remained at the same level later. Though the price of imported fishmeal has decreased from 2007 to 2008, it was difficult for the suppliers to reduce their feed prices. A certain major maker explained that the prices of other commodities such as fish oil and vitamins have been increasing.

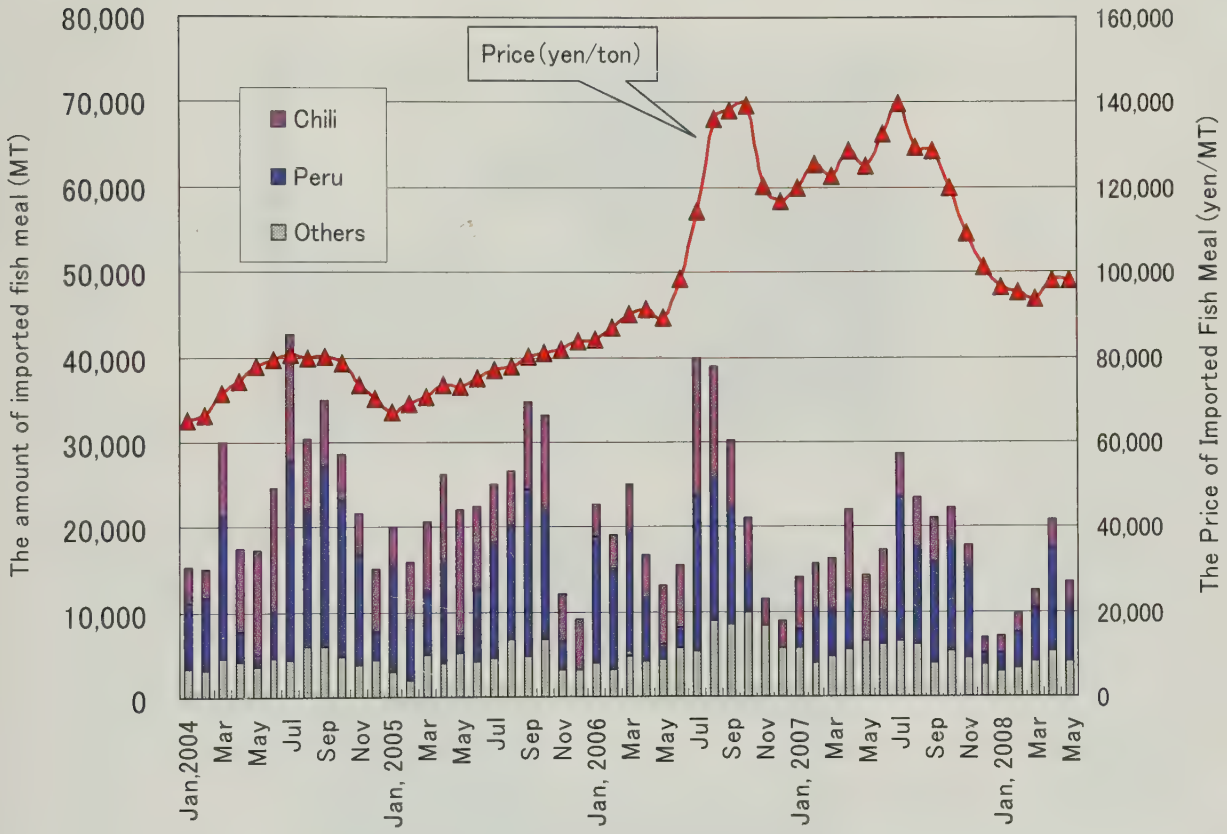


Fig.4. Amounts and prices of imported fishmeal.
Source : *2004-2008, Trade Statistics of Japan,* Ministry of Finance.

Raw feed

Raw feeds are widely utilized for yellowtail production in Japan (Fig. 2). Sardines, anchovies and jack mackerel (called large captured pelagic fishes) are low value species commonly utilized as raw feed. The catch amounts of these large capatured pelagic fishes in Japan are shown in Fig. 5. For example, 4.5 million tons of sardines were landed in 1988. At that time, sardine were a very important as raw feed for fish aquaculture. Since that time, the catch of sardines has gradually decreased until it reached a very small amount in recent years. Thus, raw feed for fish aquaculture has a tendency to be from by anchovy, jack mackerel and saury fisheries in addition to sardines. Jack mackerel is now an important species for use in raw feed (Fig. 5), however, the amount of export is gradually increasing (Fig. 6). The catch of mackerel decreased by 200,000 tons in 2007 compared with 2006, however, the amount of export didn't decrease to the same extent. It seems that the demand for low-price fishes such as mackerel has been increasing in places such as China, Korea and southeastern Asian countries. The prices obtained for raw fish for export and food are higher than for aquaculture feed.

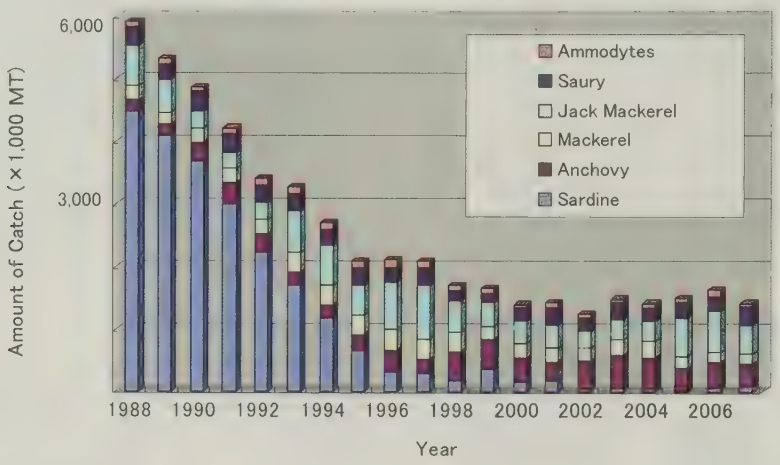


Fig. 5. Amounts of coastal catch in Japan (sardines, anchovies, mackerel, jack mackerel, saury and ammodytes).
Source : *2006-2007, Annual Statistics on Fishery and Aquaculture Production,* Statistics Department, Ministry of Agriculture, Forestry and Fisheries.

Fig. 6. Amounts of coastal catch and export of mackerel.
Source : *2006-2007, Annual Statistics on Fishery and Aquaculture Production,* Statistics Department, Ministry of Agriculture, Forestry and Fisheries. & *2001-2007, Trade Statistics of Japan,* Ministry of Finance.

Cost

The details production costs for yellowtail and red sea bream are shown in Fig. 7. The proportion of feed cost to total production one was 67% in yellowtail and 64% in red sea bream in 2006. It seems that a sudden rise in prices of mixed and raw feeds has affected the management practices of aquaculture farmers as the feed costs have increased as a percentage of total production costs. It can be assumed that fish farmers have not passed the increased cost of feed to the market prices of their products because there has been little change in the average prices of yellowtail and red sea bream since the significant increase in feed costs (Fig. 8).

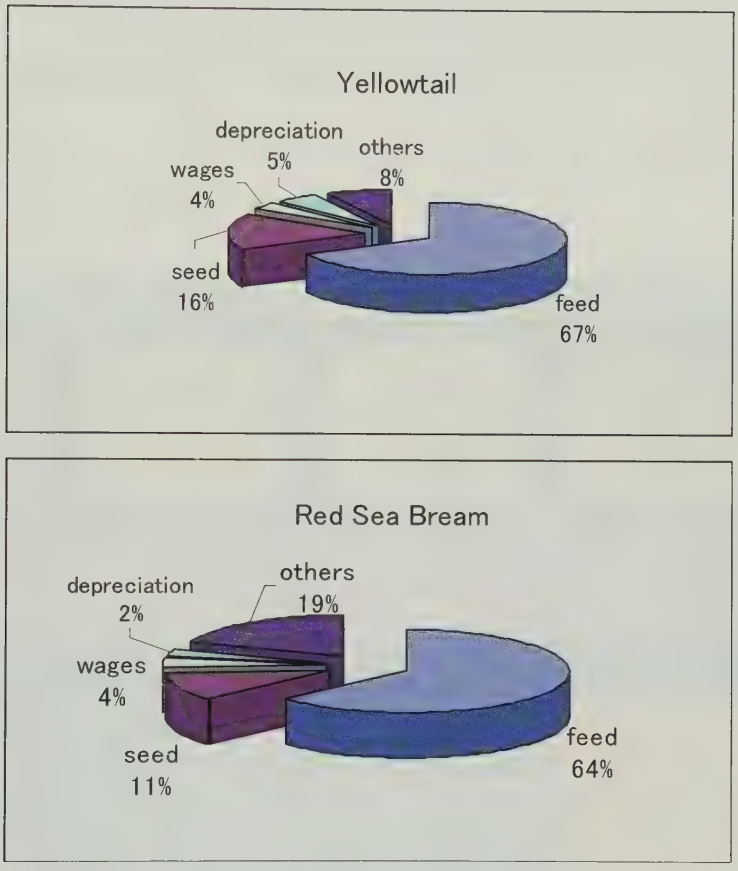


Fig.7. Details of production costs of yellowtail and red sea bream.
Source : *2006, Report of Statistical Survey on Fishery Management,* Statistics Department, Ministry of Agriculture, Forestry and Fisheries

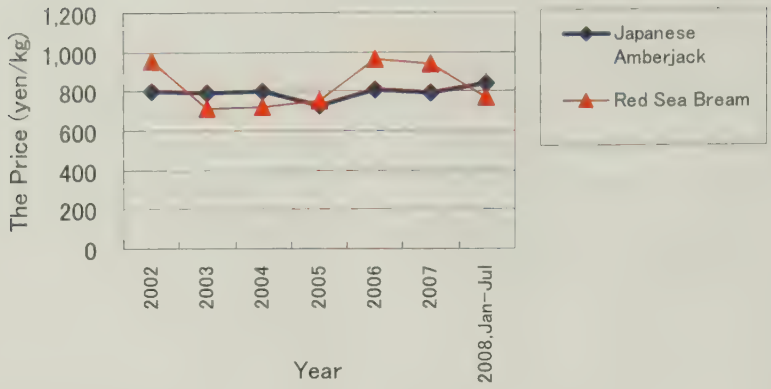
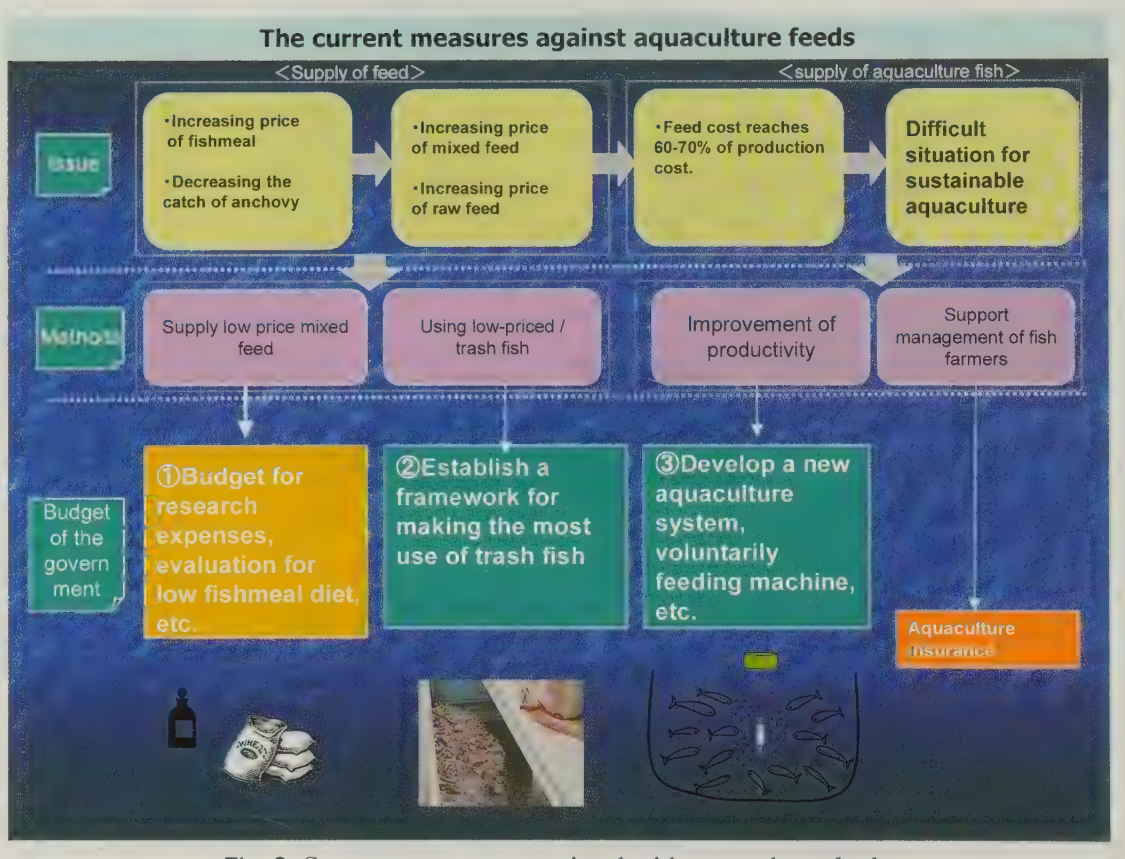


Fig. 8. Prices of aquacultured fish in the Japanese market.
Source : Monthly bulletin of Japan Sea Water Fishery Cultivation Association. No.449-No.527. (Market report of Tokyo, Yokohama, Osaka, Kobe, Hiroshima, Kitakyusyu and Fukuoka)


The current measures against aquaculture feeds
<Supply of feed>
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Issue
・Increasing price of fishmeal
・Decreasing the catch of anchovy
・Increasing price of mixed feed
・Increasing price of raw feed
・Feed cost reaches 60-70% of production cost.
Difficult situation for sustainable aquaculture
Measure
Supply low price mixed feed
Using low-priced / trash fish
Improvement of productivity
Support management of fish farmers
Budget of the govern ment
①Budget for research expenses, evaluation for low fishmeal diet, etc.
②Establish a framework for making the most use of trash fish
③Develop a new aquaculture system, voluntarily feeding machine, etc.
Aquaculture insurance

Fig. 9. Current measures associated with aquaculture feeds.

Administration policy

The Japanese government (Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries) is comprehensively and systematically promoting measures related to fisheries, including aquaculture, by developing sustainable production targets according to the Basic Plan for Fisheries Policy, which was established in 2007 based on the Basic Law on Fisheries Policy. The plan describes the means to address the issues related to aquaculture in Japan, including the promotion of creation and improvement of aquaculture grounds, prevention of diseases and introduction of new species for aquaculture, as well as improvement of the quality of aquaculture products through the improvement of breeding methods.

With respect to aquaculture feeds (Fig. 9), some laboratories have been carrying out investigations on new types of mixed feeds that can reduce costs. Studies have included evaluation of some low-cost materials such as lees (sediments) from *shochu*, which is a clear liquor distilled from sweet potatoes. In addition, studies have evaluated artificial taurine as a means of reducing the amount of fishmeal in mixed feeds. The Japanese government supports the investigations and budgets for them.

It is important for fish farmers to obtain feeds at reasonable prices. However, the prices of such feed components as fishmeal and fish oil have been increasing recently. In addition, the recent depression has beaten down the price of products. Therefore, many fish farmers are being confronted with financial difficulties that can lead to the closure of their businesses, with the consequence being reduction in aquaculture production. To address the situation, the government has promoted the mechanization of aquaculture through, for example, the use of voluntarily feeding machines which can improve the efficiency of feed utilization and provide more eco-friendly farm sites. The government is also attempting to establish a framework for providing a consistent supply of trash fish for use as feed by fish farmers.

To achieve sustainable aquaculture, we established a fisheries mutual aid system which played an important role in risk management by fish farmers. As Japan is one of the most advanced countries for aquaculture and one of the largest consuming countries of fisheries products, we are responsible for the development of sustainable aquaculture, must discharge that responsibility and we can!

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An Overview of Progress toward Developing an All Plant-based Diet for Rainbow Trout

Gibson T. GAYLORD [*1, *3], Frederic T. BARROWS [*1], Kenneth E. OVERTURF [*1], Keshun LIU [*2], and Gongshe HU [*2]

Abstract：Feeds for rainbow trout have historically contained high quantities of fish meal and fish oil as cost effective sources of both essential amino acids and fatty acids. Research results from around the world have identified ingredients to replace fish meal and fish oils, but limits still exist in completely removing these ingredients from the diet. The USDA-ARS Trout-Grains Project, officially titled "The integration of nutritional, genetic and physiological approaches to improve production efficiency of rainbow trout (*Oncorhynchus mykiss*)" was initiated with a primary goal to identify cost effective replacements for fish meal. The project team consists of two fish nutritionists, a fish geneticist, a grain chemist and a plant geneticist. This group of scientists addresses a range of issues, including selection/development of cereal grains with improved nutritional profiles, development of methods to fractionate valuable components of grains, and development of families of trout that are better able to utilize nutrients from plant-based feeds. The nutrition component of the project focuses on evaluation of new and traditional ingredients, nutrient requirements for specific life stages, and identification of nutrients in fish meal not present in plant meals. Finally we are developing a database of nutrient availability from plant-based ingredients that will have potential to supply the nutritional needs of rainbow trout in aquaculture. Some highlights of our research findings have been: 1) Taurine may be conditionally indispensable when only plant-derived protein sources are utilized. 2) Vitamin premix formulations benefit from modification when fish meal is replaced by plant derived ingredients, or when poor quality fish meals are used in extruded trout feeds. 3) Starch amylose/amylopectin ratios in energy feed ingredients will affect carbohydrate and energy digestibility. This article will present the results of laboratory and pilot scale studies of feeding fish meal-free diets to rainbow trout.

Key words：rainbow trout, feeds, alternative ingredients, plant protein

Background

The primary reason fish meal and fish oils have been used routinely in rainbow trout and many other carnivorous fish feeds is that they have been affordable sources of amino acids and fatty acids that support optimal growth, or near optimal growth, and product quality. Although these ingredients have historically been more expensive than other sources of protein or fats/oils, the high growth rates and feed efficiencies observed when fish meal and fish oil are used makes them cost effective. With increasing demands for fish meals and fish oils, along with fluctuating supplies, price volatility has increased interest in identifying and characterizing alternative sources of protein and fats. Although many

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E-mail: Gibson_Gaylord@fws.gov

animal derived protein and fats are excellent feed ingredients for rainbow trout, consumer preferences and concerns limit their inclusion in some fish feeds. Therefore, plant-derived proteins and oils have been the focus of research performed by the Trout-Grains Project team of the USDA Agricultural Research Service in Aberdeen and Hagerman, Idaho.

Approach

One aspect of evaluating the potential of plant-derived ingredients as a source of nutrients and energy has been to thoroughly define the nutrient composition of those ingredients. The digestibility and availability of nutrients and energy from an ingredient is determined by *in vivo* testing. A database of apparent digestibility coefficients for protein and energy has been produced for multiple ingredients used in fish feeds or that may have potential as substitutes to fish meals in extruded feeds (Gaylord *et al.*, 2008a). The next step is to determine the amino acid availability from these ingredients for rainbow trout (Gaylord *et al.*, 2009). Although this database covers a variety of ingredients, new ingredients and new processing techniques are continually becoming available and provide other potential sources of dietary nutrients and energy. Barrows *et al.* (2008a) demonstrated that new processing technologies developed to remove the oil from soybeans for bio-diesel production may result in a meal that can be used in trout feeds. However, differences in the availability of amino acids in this product may differ slightly from that of traditional hexane-extracted soybean meal and careful attention to available amino acid balance should be undertaken when formulating fish diets with soybean meal from bio-diesel production.

The information garnered from amino acid availability studies of individual ingredients is leading to improved diets that not only meet the available amino acid requirements of rainbow trout but also could reduce total dietary protein concentrations and improve net protein balance. Gaylord and Barrows (2009) determined that rainbow trout fed plant-protein based diets balanced on available amino acid grew equally well when crude protein levels were reduced and limiting essential amino acids were supplemented. In fact, results in our laboratory show that protein retention efficiencies were improved by reducing dietary crude protein while balancing essential amino acids without reducing growth or feed conversion efficiencies.

Increased inclusion of plant-proteins in rainbow trout feeds may require greater attention to levels of other nutrients that are abundant in fish meal. One such instance was demonstrated when Barrows *et al.* (2008b) determined that vitamin premixes could be optimized for both plant-based as opposed to fish meal-based diets and for extrusion and storage losses of vitamin availability. These refinements in vitamin levels may equate to considerable savings in feed costs while maintaining optimal health of the fish. A second consideration when using all plant-based ingredients in fish feeds is that compounds not normally deemed necessary, and previously included in the diet due high levels of fish meal, may be left out. One such compound is taurine. Taurine is an amino sulfonic acid derived from methionine via cysteine. This bioconversion has been demonstrated in rainbow trout but may be suboptimal depending on methionine supply. Gaylord *et al.* (2006) demonstrated that taurine supplementation in a plant-based diet improved trout growth and feed conversion ratios; whereas, there was no benefit from taurine supplementation of a fish meal-based diet. These benefits were observed even though the total sulfur amino acid contents of the diet were theoretically adequate. In a follow up trial, Gaylord *et al.* (2007) observed marginal gains with taurine supplementation to a plant-based diet and detrimental effects of methionine supplementation. The mechanism whereby taurine supplementation benefits rainbow trout is still unclear but its incorporation into bile salts for fat digestion may be one factor (Kim *et al.*, 2008). Potentially, other compounds found in fish meal and other animal meals may also be absent from plant-derived ingredients. Not only have these compounds not been clearly defined, but their potential benefits as constituents of feeds for trout or other carnivorous fish have not been adequately investigated. Another such compound, hydroxyproline, has been shown to be beneficial in Atlantic salmon (Aksnes *et al.*, 2008);

however, additional research is needed in this area.

In order to include cereal grains and legumes as primary protein sources for carnivorous fish, nutritional components need to be fractionated in order to both increase protein concentrations as well as remove anti-nutritional factors. Currently, protein concentrates are available from cereal grains and include wheat gluten meal, corn gluten meal, rice protein concentrate and barley protein concentrate. The digestibility of protein and energy from these products is generally high for rainbow trout although other factors may limit inclusion. In rainbow trout feeds, xanthophylls from corn gluten meal when included at levels over 10% of the diet will impart an undesirable yellow color to the fillets. The use of wheat gluten meal also may be limited in carnivorous fish diets due to its undesirable effects at high levels on pellet quality after extrusion. Legume meals such as soybean have detrimental effects when fed to trout. These effects include intestinal enteritis that limits dietary inclusion to less than 20% in trout feeds. Protein concentrates from soybeans (soy protein concentrates and soy protein isolates) do not have these limitations since the anti-nutritional factors have been removed. These products also have produced high digestibility coefficients in rainbow trout. Hence, the use of fractionation technology to remove undesirable substances in the raw materials has resulted in several nutrient-dense, feedstuffs of plant origin.

Determining the nutrient content of various fractions of cereal grains and by-products is one of the first steps in developing technologies that may lead to refined ingredients for aquafeeds. Liu *et al.* (2007) determined the phosphorus and mineral concentrations that would influence the nutritional value of ingredients from mechanical fractionation of barley. Liu (2007a, 2007b) also developed laboratory methods for abrading various layers of barley to identify layers with potentially improved nutrient profiles. Using the methods developed, Liu *et al.* (2009) further showed that dry fractionation is the method of choice for separating barleys into fractions with varying levels of protein, beta-glucan and/or starch. However, selection of a specific single or combined method is needed for achieving maximum shifts of a particular nutrient. Determination of functional lipids in differing barley fractions may also lead to improved nutritional value for fractionated cereal grains for fish feeds (Liu and Moreau, 2008).

Identification of grain by-products that may be refined for utilization as feed ingredients for fish is also progressing. Liu (2008) determined the potential of size fractionation technologies for further concentrating nutrients of interest/value in corn distillers dry grains with solubles (DDGS). Further evaluations of DDGS fractionation technologies and compositional analyses of the developed fractions will be necessary to evaluate DDGS as an ingredient for aquafeeds.

The development of plant cultivars with improved nutritional value is also underway. The successful development of low phytic acid (LPA) lines of barley to improve phosphorus availability is one example. Dorsch *et al.* (2003) identified potential low phytic acid genotypes that have been further assessed as feed ingredients for rainbow trout. Sugiura *et al.* (1999) and Overturf *et al.* (2003) quantified increased phosphorus availability from the low phytic acid varieties of barley compared to wild type barleys, and recently the 'Clearwater' variety, a hull-less low phytic acid barley, has been registered (Bregitzer *et al.*, 2008). Another nutritional trait that has been successfully screened in barley varieties is starch type (waxy vs. normal starch). The nutritional value of waxy lines of barley has been analyzed with respect to their ratio of amylose to amylopectin, and the digestibility of these starch fractions has been determined in rainbow trout (Gaylord *et al.*, 2008b). Further development of cereal grain varieties for improved nutritional value has been on-going. Hu *et al.* (2007) has been surveying genome-derived SSR markers as potential tools to aide in oat genetic research. Hu and Burton (2008) modified an enzymatic beta-glucan measurement method into a high throughput and low cost assay format. This tool will help to speed up the improvement of starch related grain qualities of cereal for better use in fish feed.

Conclusion

Fish meal is a good source of nutrients in

aquaculture feeds, but is not required by trout for optimal growth. Many opportunities exist for improvements in the nutritional value of crops for aquafeeds. Supplementing plant derived ingredients with essential nutrients allows for the complete replacement of fish meal in the diet of rainbow trout. The USDA-ARS Trout-Grains Project uses a multi-disciplinary approach to develop of plant cultivars with improved nutritional value, develop ingredient processing technologies, quantify the nutritional value of ingredients, define the nutritional requirements, and breed trout for improved performance when fed plant based diets.

References

Aksnes, A., Mundheim, H., Toppe, J., and Albrektsen, S., 2008: The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets. *Aquaculture*, **275**, 242-249.

Barrows, F. T., Gaylord, T. G., Sealey, W. M., Haas, M. J., and Stroup, R. L., 2008a: Processing soybean meal for biodiesel production; effect of a new processing method on growth performance of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **283**, 141-147.

Barrows, F. T., Gaylord, T. G., Sealey, W. M., Porter, L., and Smith, C. E., 2008b: The effect of vitamin premix in extruded plant-based and fish meal based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **283**, 148-155.

Bregitzer, P., Raboy, V., Obert, D. E., Windes, J., and Whitmore, J. C., 2008: Registration of 'Clearwater' low-phytate hulless spring barley. *J. Plant Registr.*, **2**, 1-4.

Dorsch, J. A., Cook, A., Young, K. A., Anderson, J. M., Bauman, A. T., Volkmann, C. J., Murthy, P. P. N., and Raboy, V., 2003: Seed phosphorus and inositol phosphate phenotype of barley low phytic acid genotypes. *Phytochemistry*, **62**, 691-706.

Gaylord, T. G., and Barrows, F. T., 2009: Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. *Aquaculture*, **287**, 180-184.

Gaylord, T. G., Barrows, F. T., and Rawles, S. D., 2008a: Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for rainbow trout, *Oncorhynchus mykiss*. *J. World Aquac. Soc.*, **39**, 827-834.

Gaylord, T. G., Barrows, F. T., Rawles, S. D., Liu, K., Bregitzer, P., Hang, A., Obert, D., and Morris, C., 2008b: Apparent digestibility of nutrients and energy in extruded diets from cultivars of barley and wheat selected for nutritional quality in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.*, **15**, 306-312.

Gaylord, T. G., Barrows, F. T., and Rawles, S. D., 2009: Apparent amino acid availability from feedstuffs in extruded diets for rainbow trout *Oncorhynchus mykiss*. *Aquac. Nutr.*, **16**, 400-406.

Gaylord, T. G., Barrows, F. T., Teague, A. M., Johansen, K .A., Overturf, K. E., and Shepherd, B., 2007: Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **269**, 514-524.

Gaylord, T. G., Teague, A. M., and Barrows, F. T., 2006: Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *J. World Aquac. Soc.*, **37**, 509-517.

Hu, G. and Burton, C. 2008: Modification of standard enzymatic protocol to a cost-efficient format for mixed-linkage (1 → 3, 1 → 4) - β -D-glucan measurement. *Cereal Chem.*, **85**. 648-653.

Hu, G., Jackson, E. W., and Bonman, J. M., 2007: Expansion of PCR-based marker resources in oat by surveying genome-derived SSR markers from barley and wheat. *Crop Sci.*, **47**, 2004-2012.

Kim, S. K., Matsunari, H., Nomura, K., Tanaka, H., Yokoyama, M., Murata, Y., Ishihara, K., and Takeuchi, T., 2008: Effect of dietary taurine and lipid contents on conjugated bile acid composition and growth performance of juvenile Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.*, **74**, 875-881.

Liu, K. S., 2007a: Laboratory methods to remove surface layers from cereal grains using a seed scarifier and comparison with a barley pearler. *Cereal Chem.*, **84**, 407-414.

Liu, K. S., 2007b: A modified laboratory method to

remove outer layers from cereal grains using a barley pearler. *Cereal Chem.*, **84**, 399-406.

Liu, K. S., 2008: Particle size distribution of distillers dried grains with solubles (DDGS) and relationships to compositional and color properties. *Bioresour. Technol.*, **99**, 8421-8428.

Liu, K. S. and Moreau, R. A., 2008: Concentrations of functional lipids in abraded fractions of hulless barley and effect of storage. *J. Food. Sci.*, **73**, C569-C576.

Liu, K. S., Barrows, F. T., and Obert, D., 2009: Dry fractionation methods to produce barley meals varying in protein, beta-glucan and starch contents. *J. Food Sci.*, **74**, C487-C499.

Liu, K. S., Peterson, K. L., and Raboy, V., 2007: Comparison of the phosphorus and mineral concentrations in bran and abraded kernel fractions of a normal barley (*Hordeum vulgare*) cultivar versus four low phytic acid isolines. *J. Agric. Food Chem.*, **55**, 4453-4460.

Overturf, K., Raboy, V., Cheng, Z. J., and Hardy, R. W., 2003: Mineral availability from barley low phytic acid grains in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquac. Nutr.*, **9**, 239-246.

Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M., and Hardy, R. W., 1999: Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **170**, 285-296.

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Dietary Supplementation Strategies to Improve Performance of Rainbow Trout *Oncorhynchus mykiss* Fed Plant-based Diets

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Abstract : Higher inclusion levels of plant-based feedstuffs in diets for rainbow trout necessitate identification of methods to mitigate the negative effects on fish growth and health. Thus, an increasing number of novel and re-purposed dietary supplements are becoming commercially available to counteract the anti-nutritional effects of plant-based ingredients. Specifically, probiotic bacteria, which have previously been primarily investigated for their anti-pathogenic effects in fish, are now promoted to aid in the utilization of plant-based diets and digestive tract health. Additionally, dietary supplementation of commercially produced anti-inflammatory antibodies used to reduce gastrointestinal tract inflammation in terrestrial animals may also have potential to improve digestive tract health and performance of aquatic animals fed plant-based diets. The present paper describes two studies conducted at the Hagerman Fish Culture Experiment Station that investigated the ability of dietary probiotics and anti-phospholipase A_2 antibody to improve performance of rainbow trout fed soybean meal-based diets.

Key words : rainbow trout, soybean meal, probiotics, anti-phospholipase A_2

Increased utilization of plant protein meals has been embraced as a sustainable alternative to fish meal (Barrows *et al.*, 2008; Gatlin *et al.*, 2007), with soybean meal being the most viable plant protein supplement widely available and competitively priced (Nordrum *et al.*, 2000). However, inclusion rates of soybean meal in salmonid diets are currently kept at less than 20% (Hardy, 2002). At levels of >20% reduced weight gain and feed efficiency (Olli and Krogdahl, 1995; Olli *et al.*, 1995) and pathomorphological changes in the distal intestinal epithelium with diarrhea (Rumsey *et al.*, 1994) are observed. Identification of new methods to mitigate the negative effects of soybean meal on salmonid growth and health are needed. Because the hindgut of teleost fish is known to play an important immunogenic role, and strong immune and inflammatory responses are achieved by delivering antigens to this site (Ellis, 1995) methods that target this location are of particular interest. Two such methods include dietary supplementation of probiotics or anti-inflammatory antibodies.

Probiotics have been used to treat both infectious and noninfectious enteritis in humans and other terrestrial animals (Marteau *et al.*, 2002; Marteau *et al.*, 2001) and may represent a feasible method for increasing soy utilization in soy-sensitive aquatic species. Although the majority of previous probiotics research in aquatic animals has focused on reducing disease outbreaks (reviewed by Kesarcodi-Watson *et al.*, 2008; Verschuere *et al.*, 2000), it is difficult to attribute probiotic effects to suppression of a pathogen or other beneficial effects (Kesarcodi-Watson *et al.*, 2008; Gatesoupe,

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2007). The presence and endocytosis of bacteria by enterocytes in the hindgut of larvae (Hansen and Olafsen, 1999) links the gut and its microbial flora to the development of immune responses in fish. These linkages suggest that probiotics may have the potential to alter dietary soybean antigen processing in the hindgut of first feeding fish thus altering soy sensitivity in carnivorous fish (Bakke-McKellep *et al.*, 2000)

Dietary supplementation of commercially produced anti-inflammatory antibodies used to reduce gastrointestinal tract inflammation in terrestrial animals may also have potential to improve digestive tract health and performance of aquatic animals fed plant-based diets. Recently, a commercial source of anti-phospholipase A_2 antibody has become available (Aova Technology, Madison, Wisconsin, USA) and is marketed as a dietary supplement that decreases the inflammatory response observed in the gut following ingestion of a meal (Barry and Yang, 2008). Phospholipase A_2 enzyme catalyzes the hydrolysis of the fatty acid at the sn-2 position of glycerophospholipids, in response to agonist stimulation, to generate free arachidonic acid for eicosanoid biosynthesis (Balsinde *et al.*, 2006). Research in rats suggests involvement of this cascade and specifically arachadonic acid levels on the induction heat shock proteins (Gosslau and Rensing, 2000). Heat shock proteins (HSPs) are considered to be a cell survival factor and have been shown to improve cell function in multiple models of cell injury including soybean meal-induced enteritis in salmonids. Thus alteration of phospholipase A_2 activity may provide an opportunity to alter heat shock protein expression and reduce gut inflammation in rainbow trout fed diets containing high levels of soybean meal.

Materials and methods

Probiotics study

To test the hypothesis that probiotics incorporation in rainbow trout starter diets can induce immune-mediated soybean tolerance, a two-phase experimental design was employed. In the starter phase (first feeding (0.13 ± 0.01) to 6.5 ± 0.32g $fish^{-1}$), a practical-type diet was formulated to contain 48% crude protein and 20% crude fat containing either 0 , 10 or 20% soybean meal and supplemented with or without a commercially available probiotic in a 3 X 2 factorial design. Diets were fed to four replicate tanks of fish per treatment (300 fish $tank^{-1}$; House Creek strain) for eight weeks. Trout were reared in 150L tanks supplied with 4L min^{-1} of constant temperature (14.8℃) flow-through spring water. Potentially soy tolerant rainbow trout produced by feeding probiotics and increased levels of soybean meal in starter diets as described above were then fed the industry standard level 15% soybean meal or a diet with a challenge level of 43% soybean meal during a 12 week grow-out trial. Histopathological examination was performed on the distal intestine of three randomly selected fish from each tank at the end of both the starter and grow-out trial.

Antibody study

To examine the effect of a commercially available anti-phospholipase A_2 supplement on performance of rainbow trout fed high levels of soybean meal, rainbow trout were fed a practical-type control diet formulated to contain 45% protein and 15% fat with 0, 17.5 or 35% soybean meal with and without the commercially available anti-phospholipase A_2 supplement at an inclusion level of 0.3%. Diets were fed to three replicate tanks of fish per treatment (20 fish/tank, House Creek strain) for nine weeks. Trout were reared in 80L tanks supplied with 4L/min of 14.8℃ flow-through spring water. Total RNA was isolated from liver and intestinal tissues (n=3) to detect alterations in HSP27, HSP70 and HSP90 gene expression by real time quantitative PCR; protein expression was examined by Western blot. Histopathological examination was performed on the distal intestine of three randomly selected fish from each tank.

Results and discussion

Probiotics study

The use of bacterial preparations to adapt fish to new types of nutrients represents a novel probiotic application. A beneficial effect of probiotic supplementation on growth of fish fed 20% soybean

meal was observed at the end of the starter trial (Table 1). Stimulation of the immune system is considered an important mechanism to support probiotic activity (Sealey, 2000). However, no detectable benefit of probiotic supplementation on gut health or immune status was observed in fish fed 20% soybean meal. The practical application of probiotics to improve soy utilization in trout fry diets at the levels examined in starter phase are limited given that most salmonid fry diets currently do not contain soybean meal. More important, is whether the inclusion of probiotics and/or soybean meal in the diet of first feeding trout altered soy sensitivity in a manner that would increase utilization when these fish are fed higher levels during the grow-out portion of the production trial.

Table 1. Growth performance[*1], of rainbow trout fed diets containing 0, 10 or 20% soybean meal (SBM) with or without probiotics from first feeding for 8 weeks during the starter trial.

Diet	Weight gain[*2] (% increase)	FCR[*3] (g feed g gain^{-1})	Survival (%)
Without Probiotics			
0% SBM	5383[a]	1.00	98.5
10% SBM	5277[a]	1.00	97.5
20% SBM	4714[b]	0.99	97.3
With Probiotics			
0% SBM	4938[a]	1.03	97.8
10% SBM	4959[a]	1.02	97.1
20% SBM	5069[a]	0.99	96.8
Pooled SE	123	0.02	1.01
ANOVA, Pr > F[*4]	0.0142	0.8603	
Probiotics	0.1927	0.5749	0.5088
Soy	0.0401	0.5451	0.5045
Probiotics x Soy	0.0095	0.8679	0.9923

[*1]Means of four replicate tanks (300 fish tank^{-1}).
[*2](Final weight –initial weight)/initial weight x100.
[*3]FCR = feed conversion ratio; (g dry feed g wet gain^{-1}).
[*4]Significance probability associated with the *F*-statistic.

Table 2. Growth performance [*1] of rainbow trout fed probiotics then diets containing 15 or 43% soybean meal (SBM) for 12 weeks during grow-out.

Starter Diet	Grow-out Diet	Weight gain[*2] (% increase)	FCR[*3] (g feed g gain^{-1})	Survival (%)
Without Probiotics				
0% SBM	15% SBM	1515^{A}	1.17	99
	43% SBM	1213^{B}	1.28	98
10% SBM	15% SBM	1373^{A}	1.21	97
	43% SBM	1341^{A}	1.17	98
20% SBM	15% SBM	1216^{Bb}	1.26	90^{yB}
	43% SBM	1237^{Bb}	1.26	94^{yA}
With Probiotics				
0% SBM	15% SBM	1387^{A}	1.10	99
	43% SBM	1261^{B}	1.19	98
10% SBM	15% SBM	1333^{A}	1.18	99
	43% SBM	1367^{A}	1.19	99
20% SBM	15% SBM	1408^{aA}	1.19	97^{x}
	43% SBM	1253^{bB}	1.28	99^{x}
Pooled SE		41	0.03	0.8
ANOVA, Pr > F[*4]		0.0029	0.0126	<0.0001
Probiotics		0.4884	0.0535	0.0001 W>W/O
Soy		0.0658	0.0165 0=10<20	<0.0001 0=10>20
Probiotics x Soy		0.0969	0.1711	0.0010
Grow-out Diet		0.0021 15>43	0.0248 15>43	0.1188
Soy x Grow-out Diet		0.0110	0.0593	0.0249
Probiotics x Grow-out Diet		0.6956	0.2733	0.3415
Soy x Probiotics x Grow-out Diet		0.0404	0.4707	0.8705

[*1]Means of three replicate tanks (150 fish tank^{-1}).
[*2]Final weight –initial weight/initial weight *100.
[*3]FCR = feed conversion ratio; (g dry feed g wet gain^{-1}).
[*4]Significance probability associated with the *F*-statistic.

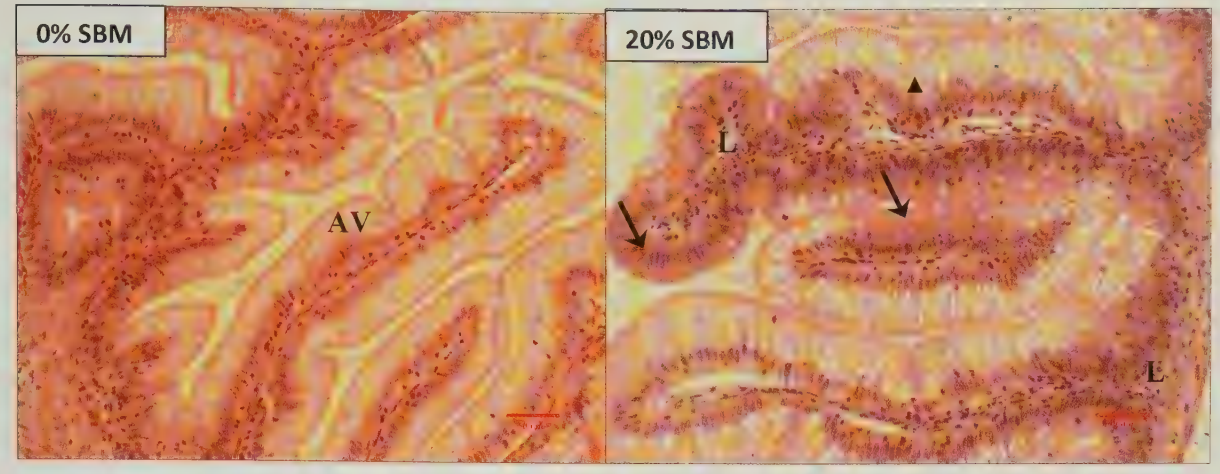

Fig. 1. Mucosal epithelium (ME) of control fish fed 0% soybean meal (SBM) during the starter trial contains numerous, finely granular absorptive vacuoles (AV' s). AV' s are lacking in some ME cells in villi of fish fed 20% SBM during the starter trial (arrows). Note large AV' s devoid of finely granular material denoted by the arrow head. Leucocytic inflammation of the lamina propria is also apparent (L) Bars equals 50 microns.

Growth performance of trout during grow-out in the same study supports a role of dietary history in soy sensitivity; both soy and probiotic inclusion in starter-diets affected fish performance in grow-out (Table 2). The beneficial probiotic effects observed during growout, however, may simply indicate an inability of fish fed 20% soybean meal in their starter diet to overcome existing tissue damage (Fig. 1). The existing damage could then result in a failure to thrive and decreased survival regardless of soybean level in grow-out diets rather than a persistent beneficial effect of probiotics that would be indicative of colonization.

Antibody study

Research by Barry and Yang (2008) examined the effects of anti-phospholipase A_2 on the growth and feed efficiency of rainbow trout fed a commercial "steelhead" diet (45% crude protein and 16% crude lipid) top-coated with 0.3% of anti-phospholipase A_2 and observed improvements compared to those fish fed the un-supplemented diet. Commercial "steelhead" diets generally contain high levels of fish meal, terrestrial animal proteins, and high quality plant protein concentrates. These diets generally include low levels of non-concentrated plant proteins to minimize the detrimental effects of plant-endogenous anti-nutritional factors; soybean meal levels, specifically, are kept below 15%. In the current study, decreased growth was observed for fish fed 35% soybean meal regardless of anti-phospholipase A_2 supplementation (Table 3). Fish fed 35% soybean meal had significantly higher feed conversion ratios than fish fed 0 or 17.5% soybean meal. Histology of the distal intestine revealed significant pathology in fish fed 35% soybean meal diets (with and without anti-phospholipase A_2) similar to that previously observed (Sealey *et al.*, 2009; Rumsey *et al.*, 1994). HSP27 and HSP90 mRNA expression was elevated in fish fed 35% soybean meal. A significant interaction was observed for HSP90 mRNA expression in that fish fed 35% soybean meal with anti-phospholipase A_2 had lower HSP90 mRNA expression that fish without supplement (Fig. 2). These data indicate feeding high levels of soybean meal alters the expression and regulation of various HSP mRNA of rainbow trout in a HSP specific manner.

Conclusions

Feeding diets with increased levels of plant protein and reduced levels of fish meal has many benefits. Soybean meal is readily available and

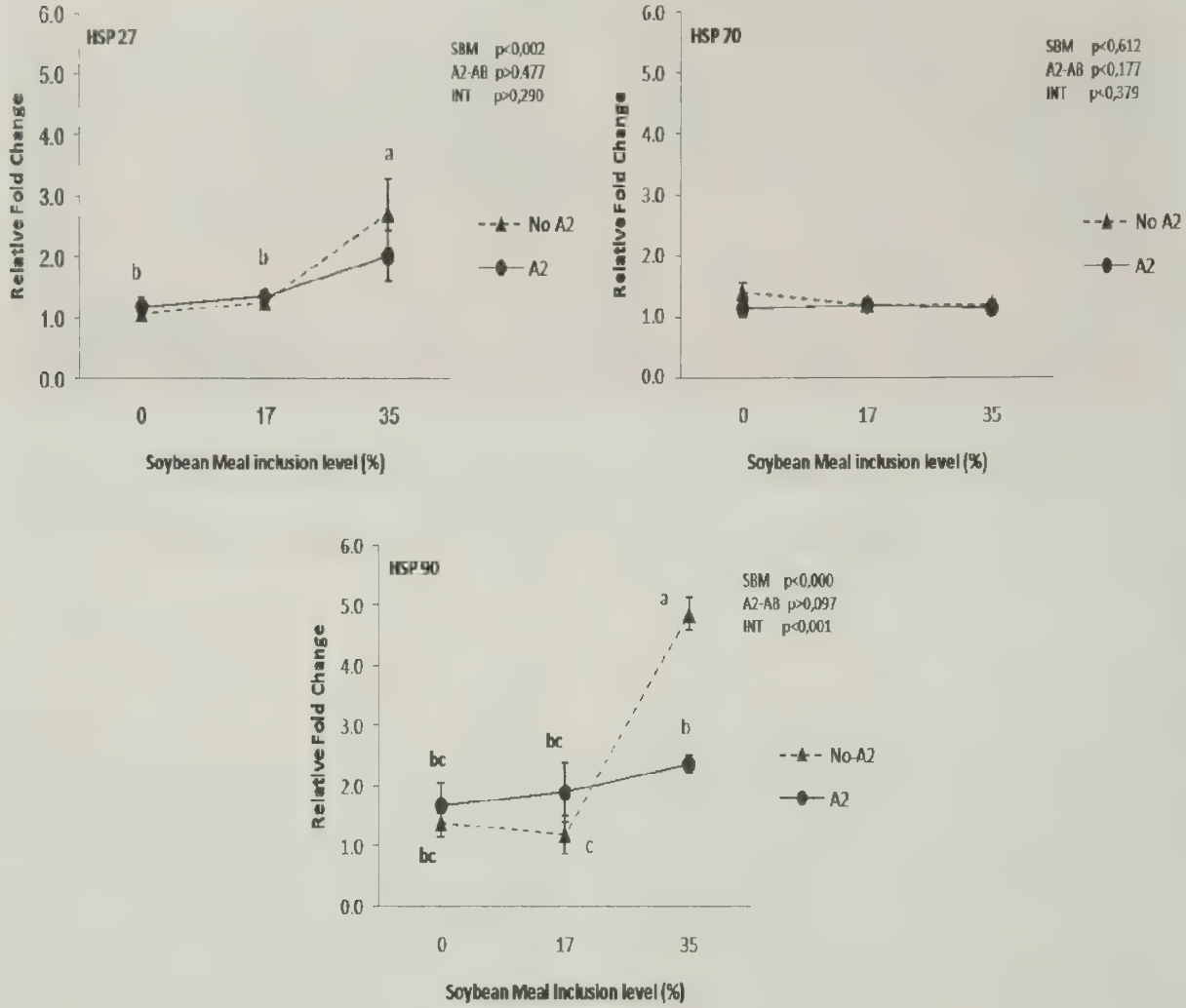

Fig. 2. Relative fold changes in the expression levels of HSP 27 - HSP 70 - HSP 90 in the small intestine of rainbow trout fed increasing levels of soybean meal (SBM) with or without the inclusion of anti-phopholipase A_2 antibody. A significance level of $P < 0.05$ was used and Fisher' s LSD tests were used to assess significant differences among treatments. All values are reported as means (n=3) ± SEM unless otherwise indicated.

cost competitive, but dietary levels are limited due to deleterious effects on growth. Results from the current studies demonstrate the potential of two different dietary strategies to improve performance of rainbow trout fed high levels of soybean meal. Specifically, the results of the probiotics study suggest that the amount of dietary soybean meal tolerated by rainbow trout can be increased in the starter phase of production by feeding probiotics. Dietary soybean meal levels may also be increased in grow-out diets by inclusion of low levels of soybean meal in the starter diets. Additionally, although dietary inclusion of anti-phospholipase A_2 at the investigated levels had only minimal effects on the pathology associated with high levels of soybean meal in rainbow trout, the beneficial effects on HSP expression suggest additional research is needed before this supplementation strategy can be completely dismissed.

Table 3. Growth performance [*1] of rainbow trout fed diets containing 0, 17.5 or 35% soybean meal (SBM) with or without anti-phospholipase A_2 for nine weeks.

Diet	Weight gain [*2] (% increase)	FCR [*3] (g feed g gain^{-1})	Survival (%)
Without antibody			
0% SBM	740^{a}	0.98^{by}	93
17.5% SBM	737^{a}	1.00^{by}	100
35% SBM	666^{b}	1.08^{ay}	98
With antibody			
0% SBM	731^{a}	1.03^{bz}	100
17.5% SBM	752^{a}	1.04^{bz}	100
35% SBM	682^{b}	1.10^{az}	98
Pooled SE	123	0.02	2.89
ANOVA, Pr > F [*4]	0.0295	0.0093	0.5705
BigFish	0.8505	0.0477	0.5314
Soy	0.0040	0.0025	0.3644
BigFish x Soy	0.8325	0.7898	0.4365

[*1] Means of three replicate tanks (20 fish/tank).

[*2] (Final average fish weight –initial average fish weight)/initial average fish weight x100.

[*3] FCR = feed conversion ratio; (g dry feed fed per g wet gain^{-1}).

[*4] Significance probability associated with the *F*-statistic.

References

Balsinde J., Pérez, R., and Balboa, M. A., 2006: Calcium-independent phospholipase A2 and apoptosis. *Biochim. Biophys. Acta,* **1761**, 1344-1350.

Bakke-McKellep, A.M., Press, C.McL., Baeverfjord, G., Krogdahl, A., Landsverk, T., 2000. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, Salmo salar L., with soybean meal-induced enteritis. *Journal of Fish Diseases,* **23**, 115-127.

Barrows F.T., Bellis, D., Krogdahl, Å., Silverstein,J. T., Herman, E. M., Sealey, W.M., Rust, M. B., and Gatlin III, D. M., 2008: Report of Plant Products in Aquafeeds Strategic Planning Workshop: An integrated interdisciplinary roadmap for increasing utilization of plant feedstuffs in diets for carnivorous fish. *Rev. Fish. Sci.*, **16(4)**, 449-455.

Barry T.P., and Yang, M., 2008: Effects of anti-phopsholipase A_2 on the growth of rainbow trout. *North Am. J. Aquac.*, **70**, 236-239.

Ellis A.E., 1995: Recent development in oral vaccine delivery systems. *Fish Pathol.*, **30**, 293-300.

Gatlin III D.M., Barrows, F. T., Bellis, D., Brown, P., Campen, J., Dabrowski, K., Gaylord, T. G., Hardy, R. W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E., Stone, D., Wilson, R., and Wurtele, E., 2007: Expanding the utilization of sustainable plant products in aquafeeds - a review. *Aquac. Res.*, **38**, 551-579.

Gatesoupe F.J., 2007: Live yeasts in the gut: Natural occurrence, dietary introduction and their effects on fish health and development. *Aquaculture*, **267**, 20-30.

Gosslau A., and Rensing., L., 2000. Induction of Hsp 68 by oxidative stress involves the lipoxygenase pathway in C6 rat glioma cells. *Brain Res.*, **864**, 114-123.

Hardy, R.W., 2002. Rainbow trout, Oncorhynchus mykiss. In:Webster, C.D., Lim, C. (Eds.), Nutrient Requirements and Feeding of Aquaculture Fish. CAB International Publishers, New York, pp. 184-202.

Hansen G.H. and Olafsen, J.A., 1999: Bacterial interactions in early life stages of marine cold water fish. *Microb. Ecol.*, **38**, 1-26.

Kesarcodi-Watson A., Kaspar, H., Lategan, M.J., and Gibson, L., 2008: Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, **274**, 1-14.

Marteau P., Seksik, P., and Jian, R., 2002: Probiotics and intestinal health effects: clinical perspective. *Br. J. Nutr.*, **88**, 51-57.

Marteu P.R., de Vrese, M., Cellier, C. J., and Schrezenmeir, J., 2001: Protection from gastrointestinal disease with the use of probiotics. *Am. J. Clin. Nutr.*, **73**, 430-436.

Nordrum S., Bakke-McKellep, A. M., Krogdahl, Å., and Buddington, R. K., 2000: Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol.*, **125B**, 317-335.

Olli J., and Krogdahl, Å., 1995: Alcohol soluble componenets of soybeans seem to reduce fat digestibility in fish-meal-based diets for Atlantic salmon, *Salmo salar* L. *Aquac. Res.*, **26**, 831-835.

Olli J., Krogdahl, Å., VabenoA., 1995: Dehulled, solvent-extracted soybean meal as a protein source in diets for Atlantic salmon, *Salmo salar* L. *Aquauc. Res.*, **26**, 167-177.

Rumsey G.L., Siwicki, A.K., Anderson, D.P., Bowser, P. R., 1994: Effect of soybean protein on serological response, non-specific defense mechanisms, growth and protein utilization in rainbow trout. *Veter. Immunol. Immunopathol.*, **41**, 323-339.

Sealey W.M. 2000. Probiotics and immunostimulants. *In* R.R. Stickney, (ed), Encyclopedia of Aquaculture, John Wiley and Sons, Inc. New York, New York.

Sealey W.M., Barrows, F.T., Smith, C. E., Overturf, K., and LaPatra, S.E., 2009: Soybean meal level and probiotics in first feeding fry diets alter the ability of rainbow trout *Oncorhynchus mykiss* to utilize high levels of soybean meal during grow-out. Aquaculture., **293**, 195-203.

Verschuere L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000: Probiotic bacteria as biological control agents in aquaculture. *Microb. Mol. Biol. Rev.*, **64**, 655-671.

Annotated Bibliography of Key Works

Bakke-McKellep A.M., Penn, M.H., Salas, P.M., Refstie, S., Sperstad, S., Landsverk, T., Ringo, E., and Krogdahl, A., 2007: Effects of dietary soybean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the telost Atlantic salmon (*Salmo salar* L.) *Br. J. Nutr.*, **97**, 699–713.

The authors explore the role of the intestinal microbiota of salmon in the pathogenesis of soybean meal-induced enteritis. A comprehensive approach is described involving identification of both descriptive and functional indices of gut health. Detailed methodology for the varied indices is provided allowing for more thorough evaluation of the effects of alternative protein sources in salmonids as well as less studied species. Results of the study indicate that soybean meal-induced enteritis is accompanied by induction of distal intestine epithelial cell responses and changes in microbiota. These results strengthen the putative linkage between gut health and microbiota and indicate that further research in this area is warranted.

Gatesoupe F.J., 2007: Live yeasts in the gut: Natural occurrence, dietary introduction and their effects on fish health and development. *Aquaculture*. **267**, 20–30.

The author summarises the present state of knowledge regarding the importance of yeast in the fish gut. The putative role of yeast is discussed in regards to both the natural occurrence and the use of "industrial" yeast in aquaculture. Functions discussed include pathogencity, colonization and competition, modulation of immune responses and alteration of host (fish) metabolism. Species, and yeast subcomponents of importance (i.e. glucans) are identified and supporting references are included.

Kesarcodi-Watson A., Kaspar, H., Lategan, M. J., and Gibson, L., 2008: Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, **274**, 1–14.

The authors review recent and historic literature regarding the use of probiotics in aquaculture. Special emphasis is placed on differences between the use of probiotics in mammalian and aquatic species. These differences illustrate and the authors described why the definition of probiotic as described by Fuller (1989), "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" must be expanded for aquatic species.
Definitions which more accurately reflect the mode of actions observed for probiotics in aquatic species are proposed. Modes of action including improving the use of feed or enhancing the nutritional value are discussed.

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Development of a New Dietary Material from Unutilized Algal Resources Using Fermentation Skills

Motoharu UCHIDA * and Tatsuo MIYOSHI *

Abstract : Decreasing fishmeal supply is a major concern for the future of the aquaculture industry. Efforts to use protein from plants to replace the fishmeal diet have been made. However, algal biomass is another candidate to replace dietary fishmeal. Marine silage (MS) is fish dietary material prepared from algae by a fermentation process. During the 34th U.S.-Japan Aquaculture Panel Symposium, we reported on a method to prepare MS from seaweed by lactic acid fermentation. The objective of this work is to report recent progress.

The fermentation of seaweeds can be performed by enzymatic saccharification by cellulase, followed by the fermentation process with the use of lactic acid bacteria. MS was first prepared with *Undaria pinnatifida* (a brown alga). In this study, we prepared MS with *Ulva* spp. (green algae) which was causing nuisance blooms in Japanese coastal waters. We also tested preparing MS from microalgae.

Developing MS is interesting from three viewpoints. First, it makes possible the conversion of algal biomass resources into dietary materials for aquaculture. Secondly, the fermentation product is acidic and long-lasting at room temperature without storage energy costs. Therefore, surplus cultured microalgae can be preserved and utilized depending on a demand. Finally, fermented materials are expected to have some useful functions that contribute to fish health. We believe that MS is a new dietary material to compensate for the lack of fishmeal and can contributes the production of high quality cultured fish.

Key words : fish diet, lactic acid fermentation, marine silage, seaweed

Recently, seafood consumption has remarkably increased in many countries. However, natural fish resources are limited in the world. Therefore, the aquaculture industry is expected to produce more and more fish. The present aquaculture system needs large quantities of fishmeal for fish feeds. However, the fishmeal supply is limited. This is a major concern for the future of the aquaculture industry. To overcome this problem, studies to use plant proteins such as soybean meal and wheat flour have been made to replace the fishmeal in fish feeds. Algal biomass is another candidate to replace fishmeal. Marine silage (MS) is fish dietary material prepared from algae by a fermentation process. During the 34th U.S.-Japan Aquaculture Panel Symposium, we reported on the method to prepare MS from seaweed by lactic acid fermentation (Uchida, 2007). The objective of this work is to report on our recent progress.

The fermentation of seaweeds can be performed by the enzymatic saccharification by cellulase, followed by the fermentation process with the use of lactic acid bacteria. MS was first prepared with *Undaria pinnatifida* (Uchida and Murata, 2002; Uchida and Murata, 2004). In the case of MS prepared from *U. pinnatifida*, algal frond tissues are decomposed during the saccharification process and the fermentation products have a size of 5～10 μ

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m in diameter. This size is suitable as a bivalve diet and *Unadaria*-MS was demonstrated to contribute to the growth of a bivalve, *Pinctada martensii* in a rearing experiment (Uchida *et al.*, 2004). MS prepared from *Ecklonia maxima* was supplemented in a formulated diet and given to red sea bream challenged with iridovirus (Uchida 2007). That study demonstrated that the MS had a function in promoting fish survival against the pathogen.

In this study, we demonstrated how to prepare MS with *Ulva* spp., which is causing nuisance blooms in Japanese coastal waters. We also examined how to prepare MS from microalgae.

Materials and Methods

Preparation of marine silage from *Ulva*

A sterile 20 L-polycarbonate tank with a screw cap (Nalgene) was used for the preparation of MS. Dried *Ulva* spp. fronds of 1.3 kg (particle size < 2mm) were dispensed into a tank containing 19.5 L of autoclaved distilled water, 26 g cellulase 12S (Yakult Pharmaceutical Ind. Co., Tokyo), 26 g macerozyme 2A (Yakult Pharmaceutical Ind. Co.), 2 g lactic acid bacteria (containing 2×10^{10} CFU of *Lactobacillus casei*, TOA Pharmaceuticals Co., Tokyo), and mixed well. The culture tank was capped, incubated at 20℃ with 70 rpm agitation (Tietech Co., Ltd, Tokyo) for 35 days, and then preserved without agitation. Frond decomposition was evaluated by measuring pecent weight proportion of algal products that passed through 100 μm nylon mesh. The number of algal particles in the culture was determined using a Coulter Multisizer (Beckman Coulter, Inc., Fullerton, CA) with a 140 μm orifice. The algal particles in the fractions of 5.8 ~ 11.5 μm in diameter were tentatively regarded as one cell product of *Ulva*-MS. Lactic acid was measured by an enzymatic method (F-kit D-/L-lactic acid, Roche Diagnostics, Basel). Lactic acid bacteria were counted on Plate Count Agar with BCP (BCP, Nissui Pharmaceutical Co., Ltd, Tokyo).

Preparation of marine silage from microalgae

Sterile 50 mL-polypropylene centrifuge tubes with a screw cap (Iwaki, Tokyo) were used for preparation of MS from microalgae. Five species of dietary microalgae: *Chrolella* sp., *Tetracelmis* sp., *Pavlova lutheri*, *Chaetoceros* sp., and *Nannochroropsis* sp. were tested. A dried product of a macroalga, *Undaria pinnatifida*, was also tested for comparison. In the cases of *Chrolella* sp. (Nissin Marinetech Co., Ltd) and *U. pinnatifida* (a gift from Riken Vitamin, Wakamidori, particle diameter <74 μm), 2g of the dried products were used for fermentation. In the cases of *Tetracelmis* sp., *P. lutheri*, and *Chaetoceros* sp., fresh cells were collected by centrifuge (3,000 rpm, 10 min.) from 200 mL cultures grown in our laboratory. Inthe case of *Nannochroropsis* sp., fresh cells were collected by centrifuge from 550 mL cultures grown in our laboratory. These algal cell samples were suspended in 40 mL 3.5% (w/v) NaCl solutions containing enzyme mixtures. Tube numbers and the enzyme compositions were: No.1 and No. 2, *U. pinnatifida* with 0.2 g cellulase; No.3, *Chrolella* sp. with 0.2 g cellulase, No.4, *Chrolella* sp. with 0.2 g cellulase and 0.2 g macerozyme; No.5, *Chrolella* sp. with 0.2 g cellulase, 0.2 g macerozyme and 0.2 g lactase Y-AO (Yakult Pharmaceutical Ind. Co.); No.6, *Tetracelmis* sp. with 0.2 g cellulose; No.7, *Tetracelmis* sp. with 0.2 g cellulase and 0.2 g macerozyme; No.8, *Tetracelmis* sp. with 0.2 g cellulase, 0.2 g macerozyme, and 0.2 g lactase; No.9, *P. lutheri* with 0.2 g cellulase; No.10, *Pavlova lutheri* with 0.2 g cellulase and 0.2 g macerozyme; No.11, *Chaetoceros* sp. with 0.2 g cellulose; No.12, *Chaetoceros* sp.with 0.2 g cellulase and 0.2 g macerozyme; No.13, *Nannochroropsis* sp. with 0.2 g cellulose; and No.14, *Nannochroropsis* sp.with 0.2 g cellulase and 0.2 g macerozyme. Four mg of a lactic acid bacteria product (containing 4×10^7 CFU of *Lactobacillus casei*) were added and incubated for nine days at 35℃ with 70 rpm agitation. Fermentation results were evaluated by measuring the pH and lactic acid content. Microscopic observation of the samples was also conducted.

Results and Discussion

Fermentation results of *Ulva* -MS

Time course change of the *Ulva* culture during fermentation is shown on Table 1. The pH value decreased from 6.87 at initial to 3.78 after 35 days of incubation, and remained constant at 3.80 during

preservation for one year. Frond decomposition proceeded from 7.6% initially to 90.5% after 35 days of incubation. One cell products were produced in the culture during incubation (Fig. 1) and the particle diameter of the products was 6.9 μm (mean value) at Day 35 (Fig. 2). The size of the one cell products became slightly smaller at 6.1 μm after one year. Lactic acid was produced at 1.99 g/L at Day 35, and further increased to 6.48 g/L after one year of preservation. Lactic acid bacteria made growth from 2.4×10^6 CFU/mL initially to 5.1×10^8 CFU/mL at Day 35. All of the colonies formed on the BCP plates were yellow colored and no contaminant bacteria were observed. The lactic acid bacteria counts gradually decreased to 1.0×10^3 CFU/mL after one year.

The present study demonstrated that lactic acid fermentation of *Ulva* spp. is possible as well as for *U. pinnatifida* as reported in a previous study. A suitable fermentation period for *Ulva* spp. was regarded as 35 days at 20℃ based on the observations that pH value, frond decomposition score, and one cell product number become constant, and lactic acid bacteria was maximized. The use of cellulase alone is effective enough to obtain the one cell products from *U. pinnatifida* (Uchida and Murata, 2002). In the case of *Ulva* spp, cellulase use alone was not effective in a laboratory study, but the use of macerozyme with cellulase was effective enough to obtain one cell products. The *Ulva*-MS could be preserved at least longer than one year at 20℃ without substantial change of characteristics.

Fermentation results of microalgae

Fermentation results for microalgae are shown in Table 2 with respect to *U. pinnatifida*. Lactic acid

Table 1. Fermentation results of *Ulva* sp.

	Incubation days (with agitation)			Preservation (without agitation)
	Day 0	Day 11	Day 35	After one year
pH	6.87	5.89	3.78	3.80
Frond decomposition (%)	7.6	60.2	90.5	100
No. of one cell products (/mL)	NT	NT	9.5×10^7	6.8×10^7
Particle diameter (mean, μm)	NT	NT	6.9	6.1
Lactic acid content (g/L)	<0.1	NT	1.99	6.48
No. of lactic acid bacteria (CFU/mL)	2.4×10^6	5.0×10^7	5.1×10^8	1.0×10^3

Frond decomposition: Per cent weight proportion of algal products that pass through a 100 μm nylone mesh aginst the total weight. NT: Not tested.

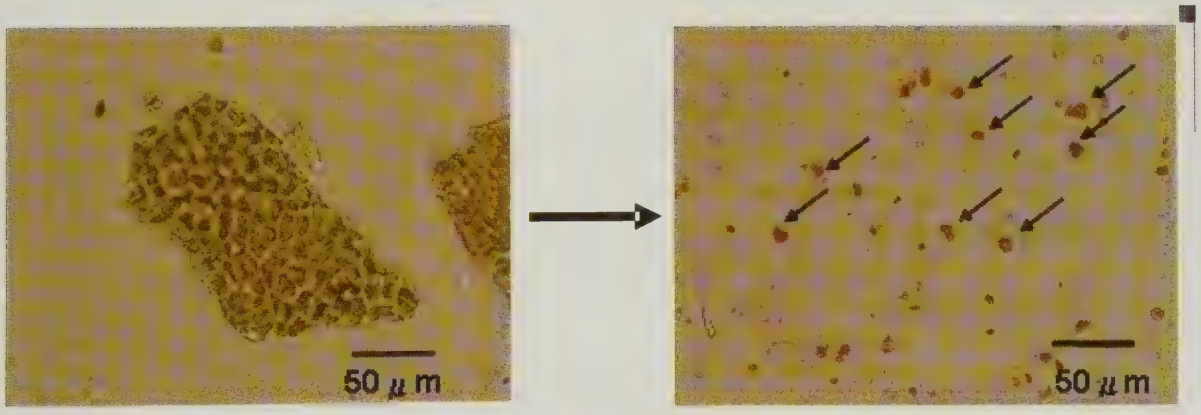

Fig. 1. Formation of one cell product from *Ulva* spp. Microscopic observation before (left) and after (right) 35 days fermentation.

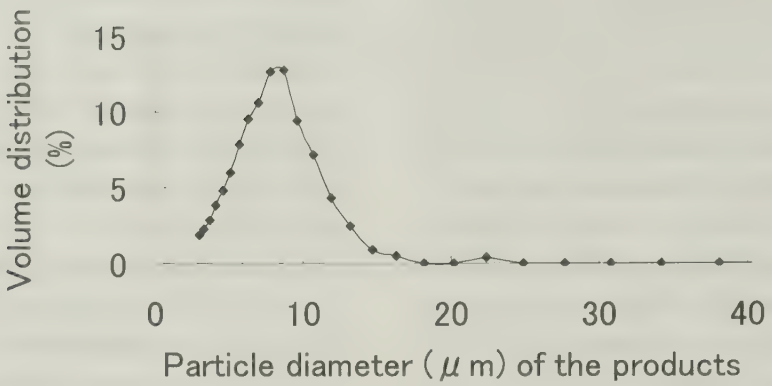

Fig. 2. Particle size distribution of *Ulva*-marine silage after 35 days fermentation.

fermentation could be performed for all the cases of microalgae as well as *U. pinnatifida* with only the use of cellulase. When comparing the results among the cases treated with cellulase alone, pH values were lower in microalgae (pH 3.32-3.80) than in the case of. *U. pinnatifida* (pH 3.87-3.90). Lactic acid contents of the cultures were 3.5 (mean), 5.4, 1.5, 1.3, and 1.3 g/L, for the cases of *U. pinnatifida, Chrolella* sp., *Tetracelmis* sp., *P. lutheri*, and *Chaetoceros* sp., respectively. Combinational use of macerozyme and lactase with cellulase resulted in an increase of lactic acid content in the cultures. Percent proportion of L-lactic acid against the total (L- and D-lactic acid) lactic acid was high (>76.2%) for most cases except that of *Chrolella* sp. In the case of *Chrolella* sp., percent proportion of L-lactic acid increased from 45.1% (with cellulase use) to 83.2% (with cellulose, macerozyme, and lactase use). Based on microscopic observations, microalgae cells looked somewhat changed in shape and were apparently partially damaged after enzymatic treatment, but the decomposition was limited and cell wall tissue remained in all cases. The color of the microalgae cells was also changed to brown, suggesting decomposition of chlorophyll content under the acidic culture conditions. Suitable enzyme product will need to be developed to obtain a cell wall-eliminated product (i.e., protoplast type marine silage).

Table 2. Fermentation results of microalgae

Tube No.	Algae samples	Enzymes			pH		Lactic acid	
		Cellulase	Maceroz.	Lactase	Initial	After ferment.	(Total, g/L)	(L-/Total, %)
1	*Undaria pinnatifida*	+			5.99	3.90	3.6	98.3
2		+			5.99	3.87	3.4	98.4
3	*Chrolella* sp.	+			6.26	3.74	5.4	45.1
4		+	+		6.32	3.64	7.9	59.2
5		+	+	+	6.35	3.29	9.6	83.2
6	*Tetracelmis* sp.	+			6.69	3.32	1.5	91.5
7		+	+		6.81	3.09	3.8	86.5
8		+	+	+	6.78	3.09	4.3	94.1
9	*Pavrova lutheri*	+			6.70	3.35	1.3	97.8
10		+	+		6.82	3.11	3.8	96.5
11	*Chaetoceros* sp.	+			8.63	3.80	1.3	76.2
12		+	+		7.83	3.31	4.0	97.0
13	*Nannochloropsis* sp	+			6.70	NT	NT	NT
14		+	+		6.64	3.15	NT	NT

NT: Not tested.

References

Uchida M. and Murata M., 2002: Fermentative preparation of single cell detritus from seaweed, *Undaria pinnatifida*, suitable as a replacement hatchery diet for unicellular algae. *Aquaculture*, **207**, 345-357.

Uchida M. and Murata M., 2004: Isolation of a lactic acid bacterium and yeast consortium from a fermented material of *Ulva* spp. (Chlorophyta). *J. Appl. Microbiol.*, **97**, 1297-1310.

Uchida M., Numaguchi K., and Murata M., 2004: Mass preparation of marine silage from *Undaria pinnatifida* and its dietary effect for young pearl oyster. *Fish. Sci.*, **70**, 456-462.

Uchida M., 2007: Preparation of marine silage and its potential for industrial use. Proceedings of the Thity-fourth U.S.-Japan Aquaculture Panel Symposium, 51-56.

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Approach to Expand the Use of Japanese Anchovy as a Feed Material : Efficacy of Thiamine Supplementation

Noriko ISHIDA [*1], Kenji NAKANISHI [*2], and Hisashi MURATA [*3]

Abstract : Three experimental diets of thiamine supplemented (TA), non-supplemented (NTA) anchovy-based moist pellets and mackerel-based moist pellets (MM) were fed to one-year-old amberjack for 20 weeks. Thiamine supplementation to anchovy pellets prevented thiamine deficiency disease observed in NTA, such as reduced levels of growth performance, plasma cholesterol and total protein, muscle fat content and disease-resistance against *Streptococcus dysgalactiase*. Furthermore, TA fish achieved the same level of growth performance, disease-resistance and meat quality as the general feed observed in MM.

Key words : Japanese anchovy, feed material, thiamine, deficiency, supplementation

The Japanese anchovy (*Engraulis japonicus*) resource is considered to be extensive but not effectively utilized. In order to resolve this issue and eventually increase the anchovy catch, we focus here on its use as an aquaculture feed material.

Anchovy is definitely the cheapest among the fish available for use in moist pellets. There is a strong demand for a cheap and readily available feed material by amberjack (*Seriola dumerili*) farmers, however anchovy is not used as a moist pellet as there is concern about the development of thiamine deficiency. The effects of thiamine deficiency were studied on yellowtail (*Seriola quinqueradiata*) in the 1970s and the problem was shown to develop in conjunction with feeding anchovies (Ishihara *et al.*, 1974). However, there have been no previous studies on thiamine deficiency in amberjack.

To increase anchovy use in inexpensive moist pellets, we investigated the development and symptoms of thiamine deficiency by feeding anchovy to 0 age group amberjack (Nakanishi *et al.*, 2007). In this paper, by feeding anchovy-based moist pellets to growing one-year-old amberjack, we observed the development of the disease, the period before its start, and its symptoms, and determined an appropriate supplement level of thiamine. In addition, the growth performance and meat quality were compared between thiamine supplemented anchovy-based moist pellets and mackerel moist pellets, a general feed currently used in amberjack farms.

Materials and Methods

Experimental diets

The ingredients used in the experimental diets were shown in Table 1. Three experimental groups of fish were fed anchovy-based moist pellets (NTA), thiamine supplemented anchovy-based moist pellets (TA) and mackerel-based moist pellets (MM), respectively. The MM diet represents the currently used feed. NTA contained 80% raw anchovy, 20% commercial feed blend and cod liver oil to adjust to the same caloric value of the other diets. TA contained 0.0013% thiamine. MM contained 80% raw mackerel, 20% commercial feed blend and cod liver oil.

The anchovy and mackerel were purchased from

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the amberjack farms in Miyazaki Prefecture. NTA and TA were prepared every four weeks and MM was prepared every eight weeks. The feeds were frozen at -20℃ before use. All the frozen experimental diets were thawed, refrigerated, and used up within three days. The proximate composition of raw anchovy and mackerel were determined in advance. Each experimental diet was adjusted to the same caloric value of 480 kcal/100 g by adding cod liver oil.

Fish and feeding trial

One-year-old amberjack were obtained from a fish farm (Miyazaki, Japan), that were transferred from China and reared starting in May 2006. Fish with an initial mean weight of 1,019 g were distributed to 2 × 2 × 2.5 m net cages. Each dietary treatment was comprised of 53 fish that were fed the experimental diets to satiation, once a day, 5 days/week for 20 weeks from June to November 2007. Bathing fish in freshwater to detach external worms and changing the cage nets to keep the living environment clean were conducted every week or every other week. The water temperature during the period was in the range 21-29℃.

Growth performance and sampling

Body weight and length of all the fish were measured initially and every four weeks. Six fish were sampled from each dietary group after 12 and 20 weeks and chemical analyses of the whole body, serum and plasma were conducted. Lipid peroxidation level, antioxidant activity, thiamine content in the liver and nutrient content in the dorsal muscle were determined.

Infection experiment

Ten fish from each dietary treatment were transferred to an indoor FRP fish tank (2 tons) after 12 and 20 weeks. Fish were injected intraperitoneally with *Streptococcus dysgalactiase* (1×10^8 CFU/fish), a major pathogen of amberjack in summer. *Streptococcus dysgalactiase* was incubated in THB medium at 37℃ for 24 h and diluted with physiological saline for injection. After 12 weeks, the first stage trial was conducted and the survival rate was observed for 14 days without feeding. After 20 weeks, the second stage trial was conducted and the fish were observed for 21 days.

Analysis methods

Proximate composition. Water contents in the diets and the fish tissues were calculated from the constant mass obtained by baking samples at 105℃. Lipid contents were measured according to the method of Bligh & Dyer (1959) using the gravimetric method. Lipid in the samples was extracted, evaporated, vacuumed and its weight measured. Crude protein content was measured as nitrogen using the Kjeldahl method. Ammonia nitrogen was formed by reacting the sample with sulfuric acid, absorbed to boric acid by the steam distillation technique and determined by neutralization analysis. Crude ash content was calculated from the constant mass obtained by incinerating samples at 600℃ in an electric furnace oven.

Thiamine concentration. The concentrations of free thiamine and its phosphate in the diets and livers were measured according to the high-performance liquid chromatography (HPLC) method of Ishii *et al.* (1979a, 1979b). Ten percent trichloroacetic acid was added to the samples, which were immediately homogenized and centrifuged. The centrifuged supernatant was transferred and extracted three times with ethyl ether. The extract was chromogenically reacted by adding 0.3 M cyanogen bromide. This solution was added to 2 N sodium hydroxide and used for HPLC analysis. A HPLC column obtained from Merck Co. was LiChrospher®-NH_2 (4×250 mm). The solvent system was acetonitrile:50 mM phosphate buffer (pH 8.4) at a ratio of 60 : 20, v/v. The spectrofluorometer was operated with an excitation wavelength set at 375 nm and the fluorescence wavelength at 430 nm. Thiamine concentration was expressed in μg per gram tissue weight.

α-Tocopherol concentration. The concentration of α-tocopherol in the diets and livers was measured according to the HPLC method of Yamauchi *et al.* (1991). Samples were saponified to obtain the unsaponifiable material. The derived

unsaponifiable material was extracted with n-hexane and analyzed by HPLC using a Zorbox-BP-NH_2 (4 × 150 mm) column (Wako Co.). The solvent system was n-hexane:isopropyl alcohol at the ratio of 200:1, v/v. The spectrofluorometer was operated with the excitation wavelength set at 295 nm and the fluorescence wavelength at 325 nm. For the internal standard material, 2,2,5,7,8-pentamethyl-6-hydroxychromane was used. The concentration of α-tocopherol was expressed in μg per gram tissue weight.

Ascorbic acid. The concentrations of ascorbic acid and dehydroascorbic acid in the diets and livers were measured in simultaneous quantification according to the HPLC method of Ito *et al.* (1995). Ascorbic acid and dehydroascorbic acid in the sample was extracted with 5% metaphosphoric acid and analyzed by HPLC. A HPLC column obtained from Shimadzu Co. was Shim-pack SCH-101H (7.9 ×300 mm). The mobile solvent was oxalic acid and 100 mM sodium hydroxide was used as a reaction solution. The ultraviolet spectrofluorometer was operated at 300 nm. The concentration of ascorbic acid and dehydroascorbic acid was expressed in μg per gram tissue weight.

Lipid peroxidation. The contents of triacylglycerol hydroperoxide (TGOOH) and phosphatidylcholine hydroperoxide (PCOOH) in the diets and livers were measured according to the chemiluminescence HPLC method of Ito *et al.* (2000). A HPLC column obtained from Shimadzu Co. was Shim-pack SIL(M) (250 × 4.6 mm). The mobile solvents was acetonitrile:methanol:water (5.5:4:0.5, v/v) and 1 μg/ml cytochrome c in 20 mM boric acid buffer containing 1% methanol (pH 10.5) was used as a chemilumigenic reagent. The active oxygen was expressed in nmol per gram tissue weight.

Thiobarbituric acid value (TBA value). The TBA value in the diets and livers was measured according to the steam distillation method of Ando & Yamauchi (1968). Malondialdehyde (MDA), lipid peroxide degradation product was reacted with thiobarbituric acid to produce red derivative. The red derivative (MA) was calculated by measuring absorbance of 532 nm. The active oxygen was expressed in μg MA per gram tissue weight.

Hematological variables and plasma biochemical property. Red blood cell content (RBC) was measured by the visual method. Hematocrit value (Ht) was obtained by centrifugation (1,200 rpm, 5 min) using hematocrit centrifuge tube. Hemoglobin concentration (Hb) was measured by cyanmethemoglobin method (Kawatsu, 1969). EC50 values of osmotic tolerance erythrocyte was expressed the NaCl concentration of 50% red cell hemolysis according to the methods as described previously in Takagi *et al.* (2006). Total plasma protein was measured using a plasma protein analyzer (Hitachi Co.). Plasma biochemical property was measured using a clinical chemistry automated analyzer (Arkray Co.).

Statistical analysis. Growth and feed performance, blood variables, osmotic tolerance in blood cell membranes, plasma biochemical analyses and hepatic lipoperoxidative levels were analyzed by ANOVA and the statistical significance of difference between treatment groups was assessed at the 5% level of probability by Tukey-Kramer test (Kramer, 1956). The results of the infection experiment were assessed by Fisher's exact test (Yanai, 2000).

Results

Experimental diets

The proximate compositions and thiamine concentrations of the experimental diets are shown in Table 1. In the proximate compositions, MM contained a higher crude protein content and less crude fat than NTA and TA. In the thiamine concentrations, NTA contained 0.2 mg/100 g fish, TA contained 1.7 mg/100 g fish and MM contained 0.7 mg/100 g fish. In terms of energy, all the diets had similar values. In the energy/crude protein ratio, MM showed the least value.

Feeding trial

Growth performance. The growth performance values for fish fed the experimental diets are shown in Table 2. No deaths from thiamine deficiency were seen during the trial. The NTA group showed

a remarkably lower specific growth rate (SGR) and feed conversion ratio (FCR) after 8 weeks and significantly lower final weight and condition factor at 20 weeks compared with the other groups. Thiamine was not added to MM, however the MM group showed constant growth, along with the lowest FCR and daily feed intake (DFI), and highest SGR. TA group also showed a constant growth rate and reached a similar final body weight to the MM group at 20 weeks.

Organ weights. The organ weights of the experimental fish are shown in Table 3. The NTA group showed the lowest liver and gut weights. On the other hand, no differences were seen between the TA and MM groups.

Hematological variables, osmolality tolerance of erythrocytes and plasma biochemical properties. The hematological variables, osmolality tolerance of erythrocytes and plasma biochemical properties in the experimental fish are shown in Table 4. At the end of the trial, the NTA group showed significantly a lower hemoglobin concentration (Hb) than the MM group. NTA group also showed the significantly lowest plasma total cholesterol and total protein levels.

Table 1. Composition and nutrient contents of experimental diets.

Diet:	NTA[*1]	TA[*2]	MM[*3]
Ingredient (%)			
Anchovy	80	80	
Mackerel			80
Commercial feed blend [※1]	20	20	20
Cod-liver oil [※2]	4.9～10.7	4.9～10.7	4.4～4.7
Thiamine	0	0.0013	0
Nutrient content (dry matter basis %)			
Crude protein	50.0	49.5	56.4
Crude fat	28.2	27.5	24.8
Sugar	11.1	12.4	8.8
Ash	10.7	10.5	10.1
Thiamine (mg/100g)	0.2	1.7	0.7
Energy (kcal/100g)	481.9	477.9	476.4
Calorie/Protein ratio[※3]	96.4	96.8	84.6

[*1] Non-thiamine supplemented anchovy-based moist pellets

[*2] Thiamine supplemented anchovy-based moist pellets

[*3] Mackerel-based moist pellets

[※1] Marubeni Nisshin Feed Co. Ltd.

[※2] Yamakei-Sangyo Co. Ltd.

[※3] Calorie(kcal/kg) to protein(% protein) ratio.

Table 2. Growth performance of amberjack fed the experimental diets for 20 weeks.

Diet	Body weight (g) Initial	Body weight (g) Final	SGR※1 (%)	CF※2	FCR※3	Daily feed intake (%)	Death (%)
NTA	1015	1642[a]	0.34	19.9[a]	6.3	1.54	1.9
TA	1034	2333[b]	0.58	22.7[b]	3.7	1.48	0.0
MM	1008	2361[b]	0.61	22.6[b]	3.0	1.40	3.8
ANOVA※4		**		**			

※1 Specific growth rate

※2 Condition factor

※3 Feed conversion ratio; g feed/g weight gain

※4 Result of two-dimensional correlation analysis.

** Significantly different ($P<0.001$)

Values are means ±standard deviation for the number of fish indicated.

Means with different superscripts within the same column are significantly different ($P<0.05$).

Table 3. Organ weights of amberjack fed the experimental diets for 20 weeks.

Diet	Liver (%) ※1	Gut (%) ※2	Spleen (%) ※3
NTA	0.6 [a]	3.9 [a]	0.16
TA	1.0 [b]	5.3 [b]	0.12
MM	1.1 [b]	5.4 [b]	0.15
ANOVA ※4	**	**	NS

※1 Liver weight (g) / Body weight (g) × 100

※2 Gut weight (g) / Body weight (g) × 100

※3 Spleen weight (g) / Body weight (g) × 100

※4 Result of two-dimensional correlation analysis.

** Significantly different ($P<0.001$)

NS, no significant difference

Values are means ±standard deviation for the number of fish indicated.

Means with different superscripts within the same column are significantly different ($P<0.05$).

Hepatic hydroperoxide levels and antioxidant activities. Hepatic hydroperoxide levels and antioxidant activities in the experimental fish are shown in Table 5. In the hepatic hydroperoxide level, the NTA group showed the significantly highest value. In terms of antioxidant activity, the NTA group showed lower ascorbic acid and dehydroascorbic acid at 12 weeks but there were no significant differences for α-tocopherol and γ-tocopherol among the groups.

Hepatic thiamine content. Hepatic thiamine contents in the experimental fish are shown in Table 6. In all groups, thiamine pyrophosphate (TPP) was dominant, accounting for 90% of the total thiamine. The NTA group showed significantly lower TPP and total thiamine than the other groups at 12 and 20 weeks. The TA and MM groups showed similar tendencies in thiamine content level and the total thiamine content of both groups showed a constant value of about 3 μg/g.

Table 4. Hematological variables, osmolality tolerance of erythrocytes, and plasma biochemical properties in the experimental amberjacks fed the experimental diets.

Diet	Red blood cell count	Hemoglobin concentration	hematocrit level	MCV※1	MCHC※2	MCH※3	EC50※4	Plasma total cholesterol	Plasma total protein
	(10^6/mm^3)	(g/dl)	(%)	(μm^3)	(%)	(pg)	(NaCl %)	(mg/dl)	(%)
Stage 1 (12 week)									
NTA	3.89	8.27	44.4	116.0	18.8	21.9	0.34	260	7.8
AM	4.17	8.53	49.2	118.5	17.4	20.6	0.33	278	8.4
MM	3.84	8.47	44.6	116.7	19.0	22.2	0.31	262	8.1
Pooled SEM	0.130	0.177	1.123	2.751	0.354	0.669	0.004	6.8	0.11
Probability	0.556	0.817	0.181	0.930	0.164	0.598	0.067	0.521	0.212
Stage 2 (20 week)									
NTA	3.46	7.71^a	46.0	134.6	16.8	22.6	0.41	241^a	7.8^a
TA	3.40	8.47^b	48.3	142.2	17.6	25.0	0.40	315^b	9.2^b
MM	3.85	8.60^b	49.3	128.3	17.5	22.5	0.38	315^b	8.8^b
Pooled SEM	0.097	0.144	0.898	2.225	0.209	0.489	0.007	5.7	0.13
Probability	0.165	0.047	0.344	0.066	0.285	0.094	0.340	<0.001	0.002
Results of ANOVA※5									
Diet	NS	NS	NS	NS	NS	NS	*	**	NS
Period	*	NS	NS	**	*	*	**	**	**
Interaction	NS	NS	NS	NS	NS	NS	NS	**	**

Values are average of 6 fish.
Different superscript letters in the same column indicate a significant difference.
※1 Mean corpuscular volume
※2 Mean corpuscular hemoglobin concentration
※3 Mean corpuscular hemoglobin
※4 NaCl concentration at EC50 for osmolality tolerance of erythrocytes
※5 Two-dimensional correlation analysis by ANOVA
** $P < 0.01$
* $P < 0.05$
NS, No significant difference

Table 5. Lipid peroxidation level and antiooxidative activity in liver of experimental amberjacks fed the experimental diets.

Diet	TGOOH※1 (nmolAO/g)	PCOOH※2 (nmolAO/g)	TBARS※3 (μgMA/g)	ASA※4 (μg/g)	D-ASA※5 (μg/g)	T-ASA※6 (μg/g)	α-Toc※7 (μg/g)	γ-Toc※8 (μg/g)
Stage 1 (12 week)								
NTA	18.7	0.09	0.16	3.5	15.8	19.3	95.3	0.02
TA	16.6	0.09	0.16	4.6	14.8	19.5	95.3	0.04
MM	16.0	0.06	0.13	4.1	15.4	19.5	114.6	0.02
Pooled SEM	0.68	0.01	0.02	0.30	0.63	0.81	8.31	0.01
Probability	0.303	0.163	0.809	0.380	0.815	0.997	0.580	0.673
Stage 2 (20 week)								
NTA	35.7^{a}	0.48	1.11	1.7	12.5	14.1^{ab}	44.0^{a}	0.05
TA	19.7^{b}	0.19	0.43	1.5	10.6	12.1^{a}	75.5^{ab}	0.00
MM	20.4^{b}	0.22	0.36	1.9	14.3	16.2^{b}	99.4^{b}	0.03
Pooled SEM	1.76	0.06	0.14	0.39	0.69	0.48	4.55	0.01
Probability	0.016	0.169	0.140	0.891	0.184	0.037	0.007	0.434
Results of ANOVA※9								
Diet	**	NS	NS	NS	NS	NS	*	NS
Period	**	**	**	**	**	**	*	NS
Interaction	*	NS	NS	NS	NS	NS	NS	NS

Values are average of 3 samples of 2 pooled fish.
Different superscript letters in the same column indicate a significant difference.
※1 Triacylglycerolhydroperoxide
※2 Phosphorylcholinehydroperoxide
※3 2-thiobarbituric acid reactive substance value
※4 Ascorbic acid
※5 Dehydroascorbic acid
※6 Total ascorbic acid
※7 α-Tocopherol
※8 γ-Tocopherol
※9 Two-dimensional correlation analysis by ANOVA
** $P < 0.01$
* $P < 0.05$
NS, No significant difference

Proximate composition of experimental fish. The proximate compositions of the experimental fish is shown in Table 7. At 12 weeks, the NTA group showed a significantly lower water content and significantly higher crude protein and crude fat content than the MM group. At 20 weeks, the NTA group showed the highest water content and lowest crude protein and crude fat levels among the three groups. The MM group showed higher crude fat and crude ash content, but those differences were small.

Infection experiment. The survival rates after being intraperitoneally injected with *Streptococcus* disease pathogen to the amberjacks fed the experimental diets are shown in Figure 1. At 12 weeks, no difference was seen among the three

Table 6. Hepatic thiamine concentrations in experimental amberjacks fed the experimental diets.

Diet	TH※1 (μg/g)	TMP※2 (μg/g)	TPP※3 (μg/g)	T-TH※4 (μg/g)
Stage 1 (12 week)				
NAM	0.09	0.11[a]	1.35[a]	1.55[a]
TAM	0.12	0.18[ab]	2.69[b]	2.99[b]
MM	0.14	0.27[b]	2.61[b]	3.03[b]
Pooled SEM	0.014	0.020	0.067	0.086
Probability	0.422	0.035	<0.001	<0.001
Stage 2 (20 week)				
NAM	0.13	0.07[a]	1.73[a]	1.93[a]
TAM	0.14	0.20[b]	2.52[b]	2.86[b]
MM	0.12	0.17[b]	2.62[b]	2.91[b]
Pooled SEM	0.01	0.01	0.03	0.04
Probability	0.528	0.013	<0.001	<0.001
Results of ANOVA※5				
Diet	NS	**	**	**
Period	NS	NS	NS	NS
Interaction	NS	NS	*	*

Values are average of 3 samples of pooled 2 fish.
Different superscript letters in the same column indicate significant difference.
※1 Free thiamine
※2 Thiamine monophosphate
※3 Thiamine pyrophosphate
※4 Total thiamine
※5 Two-dimensional correlation analysis by ANOVA

** $P < 0.01$

* $P < 0.05$

NS, No significant difference

groups. However, at 20 weeks, the NTA group showed a significantly lower survival rate than the other two groups.

Discussion

The symptoms observed in the NTA groups are considered to have been due to the deficiency of thiamine because the difference between NTA and TA was only the lack of thiamine addition. There were many differences among growth performance, biochemical analyses, and survival rates in the infection test between the NTA group from the other two groups. For the use of anchovy-based moist pellets, thiamine supplementation is considered to be absolutely necessary because the NTA group

Table 7. Proximate composition in muscle of experimental amberjacks fed the experimental diets.

Diet	Water content (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
Stage 1 (12 week)				
NAM	74.8^{b}	22.4^{a}	1.83^{a}	1.55
TAM	73.8^{ab}	22.8^{ab}	2.38^{ab}	1.56
MM	73.4^{a}	23.1^{b}	2.97^{b}	1.51
Pooled SEM	0.18	0.08	0.15	0.009
Probability	0.019	0.022	0.024	0.087
Stage 2 (20 week)				
NAM	75.4^{b}	21.9^{a}	1.64^{a}	1.52^{a}
TAM	71.5^{a}	22.8^{b}	4.75^{b}	1.52^{a}
MM	72.0^{a}	23.2^{c}	3.65^{b}	1.54^{b}
Pooled SEM	0.19	0.05	0.23	0.003
Probability	<0.001	<0.001	<0.001	0.015
Results of ANOVA※1				
Diet	**	**	**	NS
Period	**	NS	**	NS
Interaction	**	*	**	**

Values are average of 6 fish.

Different superscript letters in the same column indicate significant difference.

※1 Two-dimensional correlation analysis by ANOVA

** $P < 0.01$

* $P < 0.05$

NS, No significant difference

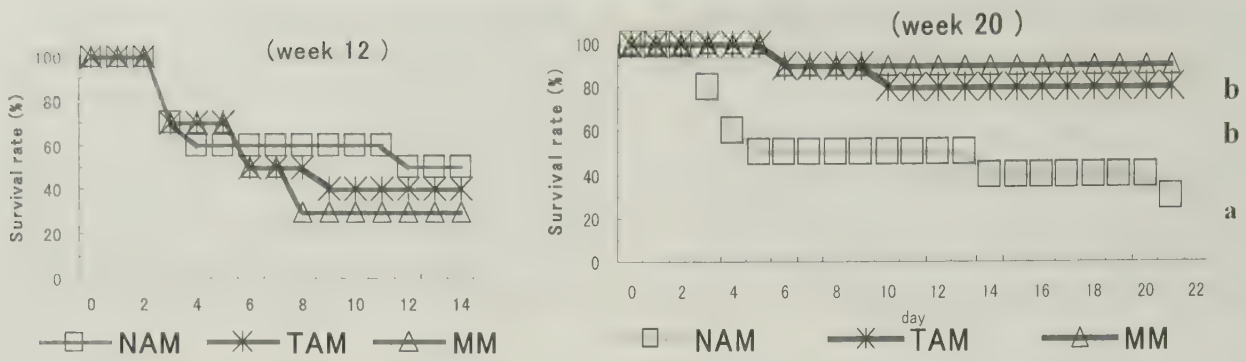

Fig. 1. The survival rate of amberjack fed the experimental diets after intraperitoneal injection at 12 weeks and 20 weeks of *Streptococcus dysgalactiae*, pathogenic to the fish. The result of infection experiment was assessed by Fisher's exact test. Different superscript letters in the same column indicate a significant difference.

at 20 weeks showed a significantly lower survival rate.

The TA group was similar to the MM group as a general feed used on commercially farms. The supplemented volume of thiamine of TA was 12.5 mg/100 g fish. The supplementation of thiamine at 12.5 mg/100 g fish is thought to prevent amberjacks fed anchovy from developing a thiamine deficiency. We also conducted another experiment with thiamine supplemented at 25 mg/100 g fish, double the concentration of the present experiment, and showed little difference between the results (not published).

On the other hand, the MM group results support the use of mackerel-based moist pellets on commercial farms. The MM was not supplemented with thiamine but the thiamine concentration of MM was similar to that of TA. In addition, the MM group showed the highest growth performance.

The TA group showed similar performance to the MM group with respect to growth performance and meat quality when the caloric values were adjusted to be equal. From the above results, we conclude that anchovy-based moist pellets should be used on commercial farms only when supplemented with thiamine at an adequate concentration.

References

Ando, N. and Yamauchi, K. 1968: Studies on oxidative rancidity in cooked meat I. A steam distillation method for the determination of TBA number of meat. *Jap. J. Zootech. Sci.*, **39**: 41-47.

Bligh, E.G. and Dyer W.J., 1959: A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911-917.

Ishihara, T., Yasuda, M., Kashiwagi, S., Akiyama, M., and Yagi. M., 1974: Studies on thiaminase I in marine fish VI. Preventive effect of thiamine on nutritional disease of yellowtail fed anchovy. *Bull. Jap. Soc. Sci. Fish.*, **40**, 775-781.

Ishii, K., Sarai, K., Sanemori, H., and Kawasaki, T., 1979a: Analysis of thiamine and its phosphate esters by high-performance liquid chromatography. *Anal. Biochem.*, **97**, 191-195.

Ishii, K., Sarai, K., Sanemori, H., and Kawasaki, T., 1979b: Concentrations of thiamine and its phosphate esters in rat tissues determined by high-performance liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **25**, 517-523.

Ito, T., Murata, H., Yasui, Y., Matsui, M., Sakai, T., and Yamauchi. K., 1995: Simultaneous determination of ascorbic acid and dehydroascorbic acid in fish tissues by high-performance liquid chromatography. *J. Chromatogr.*, **667**, 355-357.

Ito, T., Kera, A., Murata, H., Yoshida, T., Sakai, T., Yamauchi, K., Yamazaki, Y., Yamaguchi, T., and Ukawa. M., 2000: Experimentally induced bacterial hemolytic jaundice of yellowtail and oxidative stress. Nippon Suisan Gakkaishi, **66**,

50-54.

Kawatsu, H., 1969: Studies on the anemia of fish III. An example of macrocytic anemia found in brook trout, *Salvelinus fontinalis*. Bull. Freshwater Res. Lab., **19**, 161-167.

Kramer, C.Y., 1956: Extension of multiple range test to group means with unequal numbers of replications. *Biometrics*, **12**, 307-310.

Nakanishi, A., Nakamura, M., Iwata, K., Kera, A., Murata H., and Yoshida. T., 2007: Development of fish feed for healthy farmed fish I: Low cost fish feed (in Japanese). Annual Rep. Miyazaki Pref. Fish. Exp. Station, 233-243.

Takagi, S., Murata, H., Goto, T., Hayashi, M., Hatate, H., Endo, M., Yamashita, H., and Ukawa M., 2006: Hemolytic suppression roles of taurine in yellowtail *Seriola quinqueradiata* fed non-fishmeal diet based on soybean protein. *Fish. Sci.*, **72**, 546-555.

Yamauchi, K., Murata, H., Ohashi, T., Katayama, H., Pearson, A. M., Okada, T., and Yamakura. T., 1991: Effect of dietary α-tocopherol in broiler skeletal muscles and subcellular membranes and its relationship to oxidative stability. Nippon Shokuhin Kogyo Gakkaishi, **38**, 545-552.

Yanai, H., 2000: Statcel : The useful add-in forms on Excel. OMS-publishing, Saitama.

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Aquaculture Feed and Seafood Quality

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Abstract : Seafood continues to be an important protein source in many less developed countries, and over the past decade, the proportion of per capita seafood consumption produced by aquaculture has steadily grown to nearly half. Capture fisheries production has declined in recent years, and projections are for seafood landings to remain constant or decline further in the future. As a result, aquaculture production must increase to meet the expected future demand for seafood. The challenges for aquaculture in the 21st century will include not only quantity to meet increasing demand but also maintaining seafood quality for health consideration. Consumers expect safe and nutritious seafood.

Seafood quality includes characteristics such as appearance, color, texture, and taste as well as very low levels of contaminants and high levels of important nutrients. Unlike seafood harvested from the sea, the quality of aquaculture products can be controlled by many factors from the production phase to the dinner table. This report will discuss how factors such as pond management and feeding contribute to seafood quality.

Since farmers manipulate all nutrient inputs to farmed fish via the feed, the nutrient composition of farmed fish can be controlled to some degree by altering the composition of feed. For example, the color of the skin or, in salmonids, muscle tissue, can be modified by the source and quantity of carotenoid pigments, such as astaxanthin, in the feed. Replacing fish meal and oil with vegetable protein and oils or changing the oil content in fish feed will change the odor and fatty acid composition, and consequently the taste of farmed fish. Muscle lipid content can affect the texture of fish fillets as well. As far as food safety is concerned, wild fish are exposed to contaminants via their prey whereas farmed fish contaminants come from feed. Levels of contaminants in fish feeds can be closely monitored and feed formulations can be altered to reduce contaminant exposure of farmed fish to very low levels. This paper is an overview of a presentation at the workshop "Seafood Quality and Aquaculture" held in October, 2007 in Hawaii related to aquaculture feed. Manipulating the nutrient composition or boosting a selected chemical component in farmed fish products can increase the value of products and also deliver health benefits to consumers.

Key words : seafood quality, safety, feed, nutritional contents, sensory attributes

Soto (2008) recently reported that aquaculture production contributed 49% of total available seafood for human consumption in 2007. Given the fact that capture fisheries landings have not grown during the past decade and are not expected to grow in the future, the continued growth of aquaculture production was key to maintaining a constant per capita seafood supply over the same period. Because landings from capture fisheries are not expected to increase, aquaculture production must increase to meet seafood demands from an ever-increasing world population. At the same time, consumers are

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raising their expectations that seafood, from both capture fisheries and aquaculture, is both safe and nutritious.

For both farmed and wild fish, both biotic and abiotic factors contribute to fish quality starting as early as before the fertilization of eggs and continuing all the way to the dinner table. Most of the quality attributes of both wild-caught and farmed fish are the same; however many attributes can be controlled under farming conditions whereas they cannot be controlled in wild fish. For example, wild fish experience seasonal periods of food abundance and scarcity as well as variations of food type while farmed fish are fed throughout the year using feed provided by farmers.

It is generally accepted that farmed fish quality can be influenced by the formulation or composition of their feed. This is a potential advantage for farmed fish over wild fish, but one that the aquaculture industry has been slow to exploit. This report focuses on the influence of feed on seafood quality. Information presented in this report is based on the presentations at one of Aquaculture Information Program workshops "Seafood Quality and Aquaculture" held at Oceanic Institute in Hawaii in 2007 and review of current literatures.

Quality is one of the most abused words in seafood research as Alasalvar (2002) pointed out. Here we defined seafood quality as the characteristics of food that are acceptable to consumers included external factors (appearance, texture, and flavour) and internal factors (chemical, physical and microbial). Safety of seafood is also included in quality considerations.

Some species of fish have been found to contain relatively high levels of mercury and persistent organic pollutants (POPs) including PCBs, PBBs, and dioxin, and are not considered as a good quality seafood for consumption. Those contaminations can be from feed or being cultured in polluted areas, such as the Great Lakes and Puget Sound in Washington State (O' Neill *et al.*, 1998). This report will only review the quality change as a result of feeding.

Effects of feed on sensory attributes

The common sensory attributes of fish that are evaluated as part of farmed fish quality control are skin or fillet color, texture, flavor, and odor. One must keep in mind that sensory preference can vary among different cultures. Of these attributes, color, flavor and odor can be readily modified by changing feed formulation. Texture is less easily modified in fish, but there are reports that fish muscle texture can be modified by adding algae supplements to fish feeds (Nakagawa and Montgomery, 2007). Texture is thought to be modified by increasing muscle collagen and insoluble collagen fractions within muscle tissue, and by decreasing muscle lipid content. Sufficient information has been developed to provide some insight into the ways in which diet composition can influence sensory attribute of farmed fish.

Color is a critical sensory attribute of salmon and trout fillets. Consumers expect salmon fillets to be reddish-orange in color. In addition, skin color of some species of marine fish, e.g., red sea bream, porgy and similar species reared as food fish, is an importance attribute that dictates value in the market. Color in fish results from deposition of carotenoid pigments in skin and flesh. Astaxanthin and canthaxanthin, the primarily carotenoid pigments of value in food fish, cannot be synthesized by fish and therefore must be obtained from the diet both wild and farmed fish. Feeds for farmed fish must be supplemented with carotenoids to obtain pigmented skin or flesh. Carotenoid sources used in fish feeds can be obtained from natural sources, such as shrimp or krill meal, the marine algae *Haematococcus pluvialis* or Phaffia yeast, or from industrial production in the case of the major carotenoid pigments used in salmon and trout feeds, astaxanthin and canthaxanthin. A target level of carotenoid pigmentation in salmon fillets is equal to or greater than 4 μg/g wet tissue (Torrissen *et al.*, 1989). At this level of pigmentation, fillets meet consumer expectations. Higher levels of pigmentation can be achieved, but the human eye has difficulty detecting higher levels, so no additional value is associated with higher fillet carotenoid levels.

Flavor and odor are related sensory attributes, but involve different senses, namely taste and smell. Of the two, the sense of smell in humans is many orders of magnitude more developed than is the sense of taste. With respect to flavor of fisheries products detected by solely taste, very little can actually be modified by nutritional input. Actually, most of the potential for altering the flavour of fisheries products involves changing the odor. Odors in fish can be detected in uncooked products and in cooked products. Generally speaking, odors are stronger in cooked products due to the release of cellular contents which become aerosols associated with conversion of cellular water to water vapour. Many descriptors are used to characterize the flavor/odor of seafood, such as grassy, fishy, musty, metallic and so on. Trained taste panels can characterize fish products based upon descriptors.

Researchers have known for decades that certain feed ingredients alter the sensory attributes of farmed fish. Channel catfish, for example, were characterized as smelling or tasting fishier when feeds contained menhaden oil were compared to feeds containing plant oil (Dupree *et al.*, 1979). Rainbow trout were described as less fishy when sunflower oil replaced fish oil in the feed (Skonberg *et al.*, 1993). Rainbow trout fed a plant protein-based feed were described as being less sweet and having less odor intensity than trout fed a fish meal-based feed (de Francesco *et al.*, 2004). However, replacing herring oil with swine fat in rainbow trout diets did not significantly alter flavor of rainbow trout (Boggio *et al.*, 1985), suggesting that such comparisons most likely only reach significant levels when the fish oil being replaced has a strong fishy odor. Good quality herring oil smells less fishy than equivalent menhaden oil.

Efforts to alter sensory attributes of farmed fish must take into consideration the preferences of the target consuming population. For example, until recently USA consumers expected salmon fillets to be pigmented (red) and trout fillets to be unpigmented (white). This situation is beginning to change. In Europe or Japan, in contrast, rainbow trout fillets are expected to be pigmented. US consumers consistently show a strong preference for fisheries products that do not smell or taste fishy, whereas, consumers in Africa are reported to have a preference for fisheries products that have a stronger fishy component.

Effects of feed on nutritional content

Fish are high-quality sources of protein and essential amino acids, essential fatty acids, vitamins and minerals required in human diets (Nettleton and Exler, 1992). They are also relatively low in saturated fatty acids and total fat relative to other protein sources, such as beef or dairy products. The protein content of fish is relatively constant in a given species, changing slightly with fish size (Shearer, 1994). With the exception of starvation, feed input has little effect on protein content of fish muscle. The amino acid content of fish muscle tissue is excellent in terms of matching the dietary requirements of humans but it is more-or-less fixed in muscle tissue because the main proteins found in muscle, actin and myosin, have fixed amino acid compositions. Thus, protein content or amino acid profiles are not among the components in seafood that can be greatly modified by diet.

In contrast, both total lipid (fat) content and the proportion of individual fatty acids respond to changes in dietary input. Lipid content of fish, under normal growth trajectories, increases with fish size but it can be further increased when fish are fed high-energy diets such that dietary energy intake exceeds the amount required for normal metabolism, growth and activity (Grisdale-Helland and Helland, 1997; Jobling and Johansen, 2003).

Fatty acid profiles of farmed fish reflect dietary fatty acid profiles. For example, rainbow trout fed diets containing plant oils, such as canola oil, exhibit fatty acid profiles more similar to canola oil than fish oil. Similarly, trout fed diets supplemented with marine fish oil exhibit fatty acid profiles similar to marine fish oil. The fatty acid profiles of fish can be altered by changing the lipid source in the diet, but the degree of change depends upon the amount of diet (and lipid) consumed over a given period of time. If, for example, a trout was fed a feed containing primarily canola oil for the first 250 g of weight gain, then switched to a diet containing primarily fish oil for the next 250 g of weight gain, the final fatty acid

profile of the fish at 500 g total weight would reflect a combination of canola and fish oil fatty acids, similar to what would result had the fish been fed a diet containing mixture of the two oils throughout the 500 g gain feeding period. Fatty acid deposition in fish appears to follow a simple dilution model (Jobling, 2003). Fatty acid turnover in lipid depots is relatively slow, unless a fish undergoes a prolonged period of starvation or very low feed intake. Under normal fish farming conditions, there are no periods of starvation or low feed intake, at least in intensive fish farming systems.

Consumers' perception of seafood is based in part on its healthful attributes, including fillet highly unsaturated fatty acid (HUFA) content. Therefore, it is critical that farmed fish products contain levels of HUFAs that are sufficient to maintain the healthful attributes expected by the consuming public. Feeding a diet containing a combination of marine fish oil and plant oil results in lower HUFA levels in fillets than would occur had marine fish oil been the sole lipid source in the diet (Bell *et al.*, 2001). However, fillet HUFA content can be increased by feeding a finishing diet containing a high level of marine fish oil during the final phases of production. Studies with salmon and trout show that amounts of HUFAs, expressed on a g/100 g serving size, can be significantly increased by feeding a finishing diet containing marine fish oil such that the total amount of HUFAs per serving are as high or higher than that found in wild fish, although the percentage of HUFAs may be slightly lower.

Levels of some vitamins can also be increased in edible fish tissue by supplementing the diet of farmed fish. Ascorbic acid and fat-soluble vitamin levels (vitamins A, D, E and K) increase in fish tissues in proportion to dietary levels in salmonids and presumably other species of farmed fish. Levels of water-soluble vitamins (B-vitamins), with the exception of ascorbic acid, are less responsive to dietary supplementation at dietary levels higher than required levels. Excess amounts are simply excreted. Tissue levels of essential minerals, such as calcium, phosphorus, zinc and others, are maintained within a narrow physiological range in all animals, and while dietary supplementation can increase whole-body mineral levels in deficient fish, once levels reach levels corresponding to maximum tissue saturation; further supplementation does not increase tissue levels further. An exception to this is selenium, which increases in response to dietary intake due to limitations in homeostatic control when dietary intake exceeds the capacity of fish to excrete excess amounts. A similar situation occurs in the case of heavy metals, e.g. mercury, as discussed under seafood safety.

Effect of feed on safety of farmed fish products

The feeds of farmed fish can be formulated and produced to be virtually free of toxic compounds, thereby ensuring that farmed fish are among the safest products available to consumers compared to wild fish and other sources of protein.

A number of potential hazards, such as (1) methylmercury, (2) persistent organic pollutants (POPs), and (3) chemotherapeutics contamination of fish tissues occurs mainly via the feed.

Methylmercury

Mercury exists in several forms in the environment, but the form of concern in fisheries products (and all foods) is methylmercury, a methylated form of elemental mercury that is synthesized by micro-organisms and passed up the food chain to fish. Farmed fish are fed feeds that are mainly composed of plant products, such as soybean meal, corn gluten meal, and ground corn or wheat, plus fish meal, fish oil, and rendered animal products. Fish meal and fish oil are typically produced from small, short-lived pelagic fish, such as anchovies, sardines, capelin and menhaden, all unlikely to contain high levels of methylmercury. None of the plant or animal-derived ingredients is considered to be a major or even a minor source of methylmercury. Unsurprisingly, farmed fish contain negligible levels of methylmercury. The only way that fish feeds could conceivable contain significant amounts of methylmercury would be if they contained fish meal produced from trimmings of large tuna, marlin or swordfish, or if a localized population of freshwater fish from an area contaminated with methylmercury were used to produce fish meal.

POPs

POPs are organic chemicals that persist in the environment, concentrate up the food chain, and are toxin to humans, wildlife and the environment. POPs are widespread in the environment and are present in detectable levels (ppb) in nearly all fish, birds and terrestrial animals. Thus, POPs are a potential threat to humans if levels in food are high. Due to the lipophilic nature of POPs, they tend to accumulate in fatty tissue, such as the liver. Muscle tissue of most fish consumed by humans is relatively low in lipid, except for certain species, notably salmon and trout among farmed species.

Among the five species of salmon, long-lived, large species like chinook salmon (average life span 4-5 years) have higher concentrations than shorter-lived, small species like pink salmon (two-year life cycle). Compared to common food items, however, neither species is near the top of the list with respect to PCB concentration. The same holds true for farmed salmon, but, in contrast to wild salmon, farmed salmon POP intake can be lowered by diet, thereby reducing POP levels in fillets to very low levels (Ikonomou *et al.*, 2007).

PCBs and other POPs are found in trace amounts in nearly all feed ingredients, but higher levels are found in marine protein and oil, notably oil. Therefore, the nutritional approach to reducing intake of POPs in farmed fish is to reduce levels of marine protein and oil in the feed, or to use marine proteins and oils that are low in POPs as fish feed ingredients. Recently, new technology has been employed by the fish meal and oil industry to remove POPs from fish meal and oil using activated carbon (http://www.999.dk/). This approach results in very low POP levels in fish meal and oil, and virtually eliminates exposure to farmed fish from these ingredients. Past studies concerning the presence of elevated levels of POPs in farmed salmon (Hites *et al.*, 2004) do not reflect the current situation in farmed salmon (Ikonomou *et al.*, 2007). Given the known benefits of fish consumption associated with omega-3 fatty acid intake and other dietary factors, the benefits of fish consumption have been estimated to outweigh the risks for most consumers by a factor of 100 to 1 (Mozaffarian and Rimm, 2006).

Chemotherapeutant residues

To the extent that many chemotherapeutics are administered via the feed, preventing residues is a feed issue. However, it is primarily a compliance issue rather than a production issue. For chemotherapeutics that are allowed, strict rules are in place in many countries concerning withdrawal periods prior to harvest to eliminate the risk of chemotherapeutic contamination of farmed fish products. In countries having well-developed and technologically advanced aquaculture industries, such as Norway, Scotland, Canada and the USA, disease prevention is the preferred method of health management, and as such, the use of vaccines and feed additives to stimulate the immune system of farmed fish has largely replace antibiotic therapy. In this context, the use of immune stimulants and probiotics to enhance fish health deserves mention as a nutritional strategy to improve the quality of farmed fish products.

In addition to nutritional approaches to modify sensory attributes and nutritional content of farmed fish, feed supplements that produce healthy fish in top physiological and metabolic condition also must be considered in any fish farming system to ensure pre-harvest quality standards are met. The subject of feed supplements that influence health and quality of farmed fish has recently been reviewed (Nakagawa *et al.*, 2007). There are a number of feed supplements that act by modifying biochemical pathways in cellular metabolism, but there is no consensus yet concerning their efficacy. As a result, many nutritional approaches to fish quality are still being developed and evaluated in a variety of farmed fish species and culture systems. One approach that has been embraced involves the use of immunostimulants. As the name implies, immunostimulants are supplements added to feeds to stimulate the immune system of animals and fish, and thereby enhance fish health and reduce losses to disease. Immunostimulants include natural products derived from seaweed, yeast, and plants. Active ingredients in such supplements include β-glucans and nucleotides. A number of commercial immunostimulant products are available in the market and many commercial feed manufacturing

companies include these products in their feeds. Although there are a number of reports showing product efficacy, immunostimulants are not 'silver bullets' in the sense that they prevent disease in farmed fish. Rather, they are one element of many aspects of feeds and culture technology, including vaccination, that fish farmers must employ to minimize losses to disease and to ensure quality fish are produced for the consumer.

Effects of feed on storage properties

The higher the level of antioxidants in a tissue, the longer lipid oxidation is prevented. Altering the fatty acid composition of fish tissues to reduce the content of polyunsaturated fatty acids (PUFAs) and increase the level of saturated fatty acids was once proposed as a means of increasing storage stability (Cowey *et al.*, 1979) but this approach is not recommended as it would affect the primary healthful attribute of fish consumption, namely omega-3 fatty acid level.

Supplementing feeds with α-tocopherol (Vitamin E) at levels significantly higher than the dietary requirement increases tissue levels of α-tocopherol, the most effective cellular antioxidant, in a dose-response manner. In living tissue, α-tocopherol can be regenerated after losing a proton, but once fish are harvested, regeneration stops and the level of α-tocopherol decreases if conditions are favourable for the initiation step in lipid oxidation. Supplementing channel catfish feed with α-tocopherol reduced lipid oxidation in fillets during frozen storage (O' Keefe and Noble, 1978). A more elaborate study using rainbow trout was conducted by Boggio *et al.* (1985) in which both dietary α-tocopherol level and lipid source varied in feeds. Dietary levels of added α-tocopherol were 0, 50, 500 or 1500 mg kg^{-1}. The dietary requirement of trout for α-tocopherol is 30 mg kg^{-1} (Halver, 2002) and the recommended dietary level is 50 mg kg^{-1} (NRC, 1993). Dietary lipid source, either fish oil containing a high proportion of highly unsaturated fatty acids or swine fat containing a low proportion of highly unsaturated fatty acids, had no effect on frozen storage stability of fillets, whereas dietary α-tocopherol level influenced both α-tocopherol and malonaldehyde levels in fillets. Malonaldehyde, a product of oxidizing polyunsaturated fatty acids used to judge the degree of oxidation, was measured using a 2-thiobarbituric acid method. The highest level of protection from lipid oxidation was observed at the highest level of α-tocopherol supplementation.

Conclusions

The aquaculture industry must strive to improve transparency for consumers in their production techniques, and make the facts available to consumers who wish to learn more about farmed fish products. The additional challenges include reaching some agreement on target quality characteristics for established species, such as salmon and trout, for color, flavor and odor, lipid content and especially omega-3 fatty acid content of edible tissues, e.g., fillets. The consumer wants assurance that fisheries products are safe and nutritious. Only farmed products can guarantee this on a consistent and predicable basis.

Having control of the details of production of farmed fish not only offers opportunities to enhance sensory attributes of fish to fit consumer tastes, but also controls nutritional content for the characteristic of fish of most value in terms of human health, namely omega-3 fatty acid content. By using science-based nutritional approaches, farmed fish can be produced to meet target quality characteristics on a consistent basis. Altering the nutrient content of feed can improve product quality in terms of sensory attributes, content of specific nutrients, and post-harvest storage stability. The quality of farmed fish can be controlled to a large extent by feeding level, nutrient levels in feed, ingredient selection, and protein/lipid ratio of the feed fed during the latter stages of production.

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References

Alasalvar, C., 2002: Seafoods- quality, technology and nutraceutical applications. Berlin; New York: Springer, pp.1-5.

Bell, J. G., McEvoy, J., Tocher, D. R., McGhee, F., Campbell, P. J., and Sargent, J. R., 2001: Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and heptocyte fatty acid metabolism. *Nutr.*, **131**, 1535-1543.

Boggio, S. M., Hardy, R.W., Babbitt, J. K., and Brannon, E.L., 1985: The influence of dietary lipid source and alpha-tocopheryl acetate level on product quality of rainbow trout (*Salmo gairdneri*). Aquaculture, **51**, 13-24.

Cowey, C. B., Adron, J. W., Hardy, R., Smith, J. G. M., and Walton, M. J., 1979: Utilization by rainbow trout of diets containing partially rendered hide fleshings. *Aquaculture*, **16**, 199-209.

De Francesco, M., Giuliana, P., Medale, F., Lupi, P., Kaushik, S., and Poli, B.M., 2004: Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). Aquaculture, **236**, 413-429.

Dupree, H. K., Gauglitz, E. J., and Houle, C. R., 1979: Effects of dietary lipids on the growth and acceptability (flavour) of channel catfish (*Ictalurus punctatus*). Pages 87-103 *in* J.E. Halver and K. Tiews, editors. Finfish Nutrition and Fishfeeds Technology, Heenemann, Berlin.

Grisdale-Helland, B., and Helland, S. J., 1997: Replacement of protein by fat and carbohydrate diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage. *Aquaculture*, **152**, 167-180.

Halver, J. E., 2002: Fish Nutrition, 3rd Edition. Academic Press , New York, pp. 61-141.

Hites, R. A., Foran, J. A., Carpenter, D. O., Hamilton, M. C., Knuth, B. A., and Schwager, S. J., 2004: Global assessment of organic contaminants in farmed salmon. *Science*, **303**, 226-229. Available at http://www.sciencemag.org/cgi/content/full/303/5655/226.

Ikonomou, M. G., Higgs, D. A., Gibbs, M., Oakes, J., Skura, B., McKinley, S., Balfry, S. K., Jones, S., Withler, R., and Dubetz C., 2007: Flesh quality of market-size farmed and wild British Columbia salmon. *Environ. Sci. Techn.*, **41**, 437-443. Abstract available at http://pubs.acs.org/cgi-bin/abstract.cgi/esthag/2007/41/i02/abs/es060409+.html.

Jobling, M., 2003: Do changes in Atlantic salmon, *Salmo salar* L.. fillet fatty acids following a dietary switch represent wash-out or dilution? Test of a dilution model and its application. *Aquac. Res.*, **34**, 1215-1221.

Jobling, M., and Johansen, S. J. S., 2003: Fat distribution in Atlantic salmon *Salmo salar* L. in relation to body size and feeding regime. *Aquac. Res.*, **34**, 311-316.

Mozaffarian, D., and Rimm, E. B., 2006: Fish intake, contaminants, and human health: Evaluating the risks and the benefits. *J. Amer. Med. Assn.*, **296**, 1885-1899. Abstract available at http://jama.ama-assn.org/cgi/content/short/296/15/1885.

Nakagawa, H., and Montgomery, W.L., 2007: Dietary supplements for the health and quality of cultured fish. In "Dietary supplements for the health and quality of cultured fish." (edited by Nakagawa, H., Sato, M., and Gatlin III, D.M.), CAB International, Oxon, pp. 133-167.

Nakagawa, H., Sato, M., and Gatlin III, D.M., 2007: Dietary supplements for the health and quality of cultured fish. CAB International, Oxon, 244 pp.

National Research Council (NRC), 1993: Nutrient Requirements of Fish. National Academy Press, Washington, D.C. 114 pp.

Nettleton, J.A., and Exler, J., 1992: Nutrients in wild and farmed fish and shellfish. *J. Food Sci.*, **57**, 257-260.

O' Keefe, T. M., and Noble, R., 1978: Storage stability of channel catfish (*Ictalurus punctatis*) in relation to dietary level of α-tocopherol. *J. Fish. Res. Bd. Canada*, **35**, 457-460.

O' Neill, S.M., West, J. E., and Hoeman, J.C.,

1998: Spatial trends in the concentration of polychlorinated biphenyls (PCBs) in chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in Puget Sound and factors affecting PCB accumulation: results from the Puget Sound monitoring program. http://www.psat.wa.gov/Publications/98_proceedings/pdfs/2b_oneill.pdf.

Shearer, K. D., 1994: Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*, **199**, 63-88.

Skonberg, D.I., Rasco, B.A., and Dong, F.M., 1993: Effects of feeding high monounsaturated sunflower oil diets on sensory attributes of salmonid fillets. *J. Aqua. Food Prod. Tech.*, **2**, 117-133.

Soto, D., 2008: Overview of world aquaculture: trends and future prospects. Presented at Workshop: Developing a Sustainable Aquaculture Industry in the Azores. June 2-4, 2008 Azores, Portugal.

Torrissen, O. J., Hardy, R. W., and Shearer, K. D., 1989: Pigmentation of salmonids - Carotenoid deposition and metabolism. *Rev. Aquat. Sci.*, **1**, 209-225.

Annotated Bibliography of Key Works

Nakagawa, H., Sato, M., and Gatlin III, D.M., (editors), 2007: Dietary supplements for the health and quality of cultured fish. CAB International, Oxon, 244 pp.

This is the most recent book we found addresses current information on the effects of nutrients on health and quality of cultured fish. The main contents include Quality and health of cultured fish, Evaluation of quality of cultured fish, Fish health assessment, Essential nutrients, Vitamins, Amino acids, peptides, Lipids, Minerals, Natural substances, Microorganisms, Terrestrial plants, Algae, Chitin, Plant saponins, Nucleotides, Future strategies, Nutrinogenomics. We recommend this book for additional reading.

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Quality Control of Cultured Fish by Feed Supplements

Heisuke NAKAGAWA *

Abstract : The importance of algae, chitin and laurate that are widely ingested by a variety of wild fish was confirmed as feed supplement in cultured fish. The substance was supplemented to the diets of ayu, red seabream and black seabream at the same level found in the stomach content of wild fish. The dietary supplements commonly improved physiological condition such as acceleration of lipolysis activity and protein assimilation, depression of adipocytes, resistance to air-dipping, and activation of liver function, without depression of growth.

Key words : dietary supplements, fish quality, lipid metabolism, protein metabolism, vitality

Advancements in fish nutrition have progressed considerably with regard to growth performance. However, much concern has arisen over the quality of fish flesh and health in aquaculture. In response, a variety of feed supplements have been investigated with respect to their effects on physiological condition and the quality of cultured fish.

Materials and Methods

Fish and rearing condition

Prior to the experiment, organisms in the stomach contents of wild fish were analyzed. The supplemented levels of ingredients in the experimental diets were decided based on the stomach contents in wild fish. Fish used for the feeding experiment were 0-year ayu (*Plecoglossus altivelis*), black seabream (*Acanthopagrus schlegelii*) and red seabream (*Pagrus major*). Groups of experimental fish were fed either a control diet (not supplemented) or the control diet supplemented with algae (<5% in dry diet), chitin (10% in wet diet) or lauric acid (0.5% of ethyl laurate in dry diet).

Biological measurements

All the fish in each replicate treatment group were weighed individually. The whole muscle, liver and adipocyte were obtained from 20 fish of each group. Adipocyte diameter was histologically measured in 50 cells per fish.

Vitality

For comparing liver function, fish were dipped in water containing anesthetic at a concentration of 0.1% for 50 sec. Then the fish were transferred to oxygen-saturated fresh seawater and recovery time was recorded. For air-dipping resistance, fish were exposed to air-dipping for 5 min and returned to oxygen-saturated seawater. Recovery time from was recorded.

Biochemical measurements

The feed, muscle, liver and adipocytes in the intraperitoneal cavity were frozen immediately after sacrifice of the fish and kept in a freezer. Lipid was extracted with methanol-chloroform. The lipid class composition and fatty acid composition were analyzed by an Iatroscan and a gas chromatograph.

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Results and Discussion

Micro- and macro-algae

A variety of wild fishes ingest algae, including some primarily carnivorous species. The primary foods of wild ayu are benthic blue-green algae and diatoms in rivers. *Chlorella*-extract as a feed supplement affects physiological characteristics such as lipid metabolism, disease resistance, carcass quality and vitality in ayu and yellowtail (*Seriola quinqueradiata*). In the giant prawn (*Macrobrachium rosenbergii*), dietary *Spirulina* elevated growth performance and feed utilization over ranges of supplementation of 5–20% of the diet.

Although lipid content in the intraperitoneal fat body of cultured ayu was almost 98%, *Chlorella*-extract supplementation reduced the value to 66.7%. *In vitro* lipolysis activity was superior in the *Chlorella*-extract (1%) fed group, suggesting activation of lipid utilization to energy prior to muscle protein consumption. *Chlorella*-extract suppressed body weight loss caused by starvation in ayu, because the reserved lipid decreased remarkably and consumption of muscle protein might have been suppressed.

The *Chlorella*-extract distinctly improved tolerance of hypoxic conditions and liver function. The preventive action exerted by *Chlorella*-extract might involve some internal barrier to infectious disease, such as an inflammatory response and an increase in the number of phagocytes, rather than an immediate effect on the disease as an antibiotic substance.

Spirulina supplementation correlated with marked increases in some key substances for β-oxidation of fatty acids, including hepatic free carnitine and long-chain acylcarnitine, and carnitine palmitoyltransferase activity. Red seabream fed a diet supplemented with *Spirulina* at a 2% level exhibited elevated protein synthesis, and the stroma (connective tissue) fraction was significantly increased. The muscle protein composition measured by solubility approached that of wild fish. *Spirulina* increased total muscle collagen; the collagen fractions soluble at 20℃ and 70℃ decreased, but the insoluble collagen increased.

The addition of a very small amount of algal meal has produced a significant increase in the growth and feed utilization of a variety of fish such as, red seabream, Japanese flounder (*Paralichthys olivaceus*), yellowtail (*Seriola quinqueradiata*), ayu, rockfish (*Sebastes schlegeli*), nibbler (*Girella punctata*), and snakehead (*Channa striatus*). The optimum feed efficiency and protein efficiency were attained in black seabream when the supplementation level of *Ulva* meal was 2.5–5.0% of the diet. Body weight loss of black seabream during wintering was minimized with supplementation of *Ulva* meal. Growth of Japanese flounder was maximized with *Ulva* at 2% of the diet. Algae also affect other growth indicators when used as dietary supplements.

Supplementation with *Ascophyllum*, *Porphyra*, and *Ulva* at 3–5% in prepared diets elevated muscle RNA/DNA ratio (protein synthetic activity) and suppressed acid protease activity (protein catabolism) in red seabream, providing biochemical proof of growth. The effect on growth was due to an acceleration of nutrient absorption by dietary algae.

The addition of a small amount of algal meal to the fish diet can exert considerable influences on carcass quality. While micro-algae generally decrease accumulation of lipid in the muscle, macro-algae often induce an increase in muscle lipid. While muscle lipid of the control group of young yellowtail was 1.6%, supplementation of *Laminaria digitata* at 1% increased muscle lipid to 4.8%. Similar tendencies for increased muscle lipid occurred with supplementation of *Undaria pinnatifida* and *Ascophyllum nodosum* in the diet of red seabream and with *Ulva* in black seabream. Sensory evaluation of fish meat showed that supplementation of macro-algae in the diet generally improved taste and quality as a whole. Under imbalanced nutritional conditions and inadequate feeding regimes (overfeeding, inadequate feeding frequency, etc.), muscle protein may be consumed in place of lipid reserves. Fish reared under such conditions cannot endure food shortages.

Supplementation of *Ulva* meal to a red seabream diet or *Laminaria* meal to a yellowtail diet increased consumption of lipid reserves and suppressed muscle protein consumption. Wild red seabream and black seabream ingest algae in nature, and the proportion of algae ingested increases before wintering. The

ingestion of algae before wintering could help to activate lipid reserves for energy during the winter period.

Stressors during fish rearing such as high rearing density, low dissolved oxygen, water pollution, and nutritional imbalances can depress disease resistance. Resistance to low-oxygen levels was higher in algae-fed red seabream, black seabream, and rockfish. Many algal species are likely to improve vitality in a similar way, because they contain a wide array of macronutrients, micronutrients, and other components.

Supplementation of *Ulva* meal to a prepared diet at 5% elevated phagocytosis in black seabream. Effects of similar supplementation with *Ulva* were found on numbers of lymphocytes and granulocytes, agglutinin titer, and hemolytic and bactericidal activities in red seabream. In addition, bactericidal activity after immunization was enhanced. A nutritional disease involving retardation of growth and high mortality in young yellowtail is caused by continuously feeding on sardine (*Sardinops melanocticta*). The disease was reduced by supplementation of 0.5% of *Laminaria digitata*. Simultaneous addition of a vitamin mixture enhanced the effect, suggesting a synergistic effect of algae and certain vitamins. Atlantic salmon (*Salmo salar*) fed on a diet supplemented with alginate had high survival and complete hemolytic activity after challenging by *Aeromonas salmonicida*.

Chitin

Crustaceans are important and often the major food organisms of larval fish and contain considerable amount of chitin in the digesta (2.3–9.3% in dry weight). The growth, feed efficiency and survival rate were improved by adding dietary chitin (10% in a purified moist diet). The relative intestine length is an indicator of the diversified use of food and nutrients. The intestine in the chitin group was significantly longer than in the control group. The long intestine presumably extended evacuation time of food in the gut by increasing surface area. Consequently, growth performance would be improved. The dietary chitin decreased the lipid content and size of lipid droplets in the liver. Low lipid reserves was mainly due to the reduction of the adipocytes accompanied by a reduction in the adipocyte diameter. Reduction of the liver oil globules and the adipocyte size might suggest suppression of lipogenesis and activation of lipolysis. Suppression of body weight loss by starvation in the chitin group could be due to sparing the consumption of muscle protein.

Dietary chitin significantly reduced the impact of air-dipping and improved the recovery time from the succumbed condition. During the treatment, 50% of the fish died in the control group, while the chitin group recovered completely.

Lauric acid

A small amount of lauric acid was found in the stomach content of wild black seabream. Although dietary laurate did not affect growth, survival, or feed efficiency, muscle ratio was significantly increased when it was present. In addition, hepatosomatic index, adipocyte ratio, and adipocyte diameter were significantly reduced by laurate supplementation. Hepatic lipid was significantly lower; almost half that of the control group. Dietary laurate markedly depressed triglycerides, although free fatty acids and phospholipids were hardly influenced.

Dietary laurate significantly reduced the impact of air-dipping and quickened the recovery time. In the liver function test, the survival from the anesthetic condition was only 40% in the control group, but fish of the laurate group recovered completely.

Feeding laurate led to a proportional decrease of monoenes and slight increase in n-3 fatty acids in the hepatopancreas. While lauric acid was not found in the organs of the control group, lauric acid and DHA slightly increased in the heart, brain, and eye in the laurate group. Dietary laurate might account for the absorption and/or incorporation of DHA into the fish body.

References

Ji H., Om, A.D., Umino, T., Yoshimatsu, T., Hayashi, M., Nakagawa, H., Asano, M., and Nakagawa, A., 2005: Effect of dietary laurate on lipid accumulation and vitality in juvenile *Pagrus major*. *J. Fish. China,* **29**, 804–810.

Nakagawa H., Sato, M., and Gatlin III, D.M. (eds.), 2007. Dietary Supplements for the Health and Quality of Cultured Fish, 1st ed. CABI Publisher, UK, p. 133–167 and 168–177.

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Protein Production Advantages in the Face of Increasing Feed Costs: Identifying Opportunities within the Aquaculture Industry

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Abstract：This research provides an overview of the recent price trends of key feed ingredients. This includes a discussion of the current and future trends in production and prices for both agricultural (soybeans, corn) as well as marine-based (fishmeal, fish oil) sources. The focus then turns to a discussion of feed ingredients and feed costs as they relate to the production of key proteins, including both agriculture and aquaculture sources of production (e.g. broilers, shrimp, salmon, and catfish). It is then argued that the aquaculture sector is in a strong position to provide quality protein at competitive prices despite increases in raw feed materials prices. A critical factor is the degree to which the aquaculture sector, and further, certain species within the aquaculture sector, can substitute across key feed ingredients. Substitution gives farmers the opportunity to reduce feed costs in the face of rising feed prices by allowing them to find feed combinations that are both cost effective and nutritionally sufficient. Further, nutritional knowledge allows farmers the ability to take advantage of arbitrage opportunities when the relative prices of key ingredients change. Thus, aquaculture has the opportunity to manage feed costs in manner that is cost effective while also maintaining high quality protein production.

Keywords：aquaculture, fisheries, fish meal, fish oil, sustainability

Aquaculture has been the fastest growing food sector over the past two decades and this steady and rising growth in the aquaculture sector is forecast to continue into the future (Anderson, 2003). This growth will in turn fuel increased demand for key raw materials for use in aquaculture feeds. These key ingredients include both agricultural (soybeans, corn) as well as marine based (fishmeal, fish oil) sources. The prices of these key feed ingredients have increased significantly over the past few years (Fig. 1). Not only have prices increased but the volatility in prices has increased as well.

Key drivers of recent price trends

Both supply and demand side drivers have influenced the recent price fluctuations. On the supply side, weather-related events including droughts in Australia have affected agricultural production, and in particular global wheat prices, while El Nino and La Nina events have influenced fish meal and fish oil landings and prices. On the demand side, growing global populations have increased the demand for grains, both as a commodity for direct human consumption and as a feed input in the production of animal proteins (pigs, chickens, and aquaculture). The emergence of economies such as China and India has also increased the demand for key feed ingredients as these populations not only grow in size, but also in wealth. More wealth allows these economies to not only consume more food but also, to consume a more protein-based diet. The aforementioned supply and demand side drivers are drivers that have

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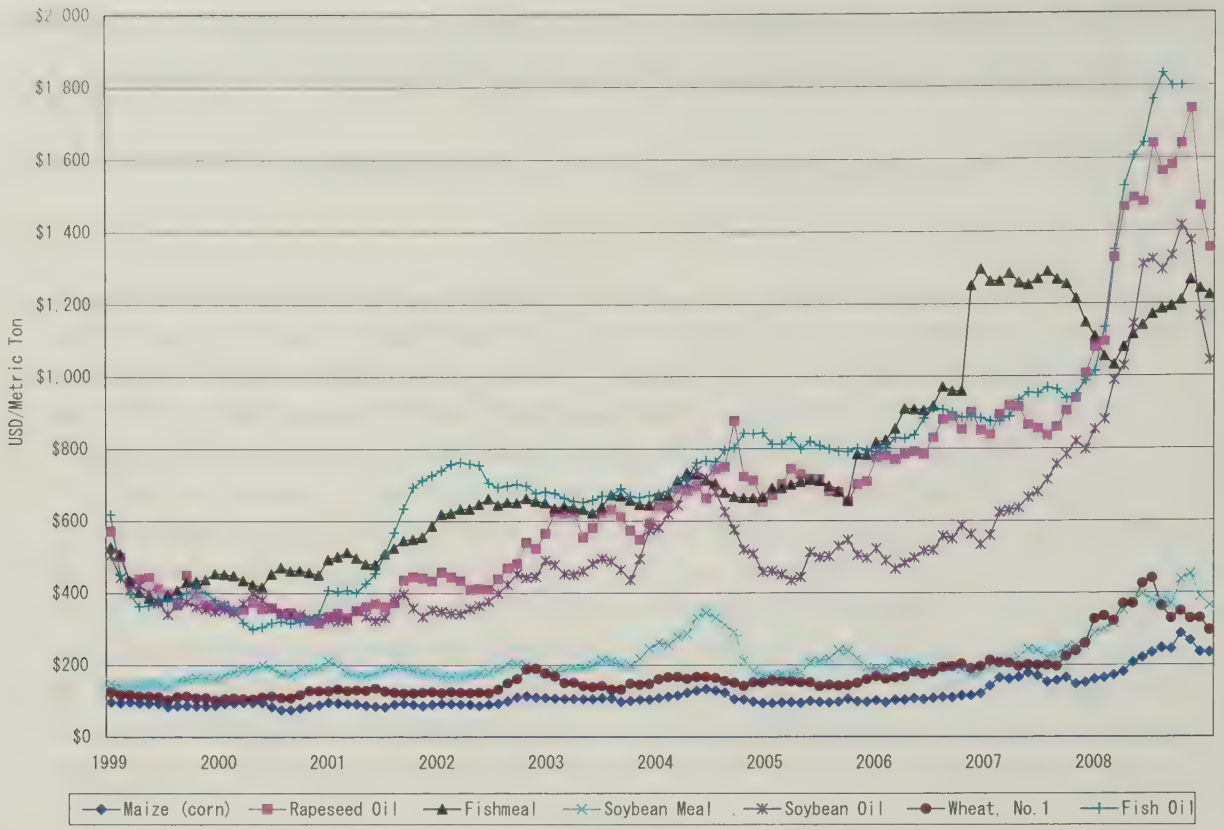

Fig. 1. Real price trends for key feed ingredients (US dollars per metric ton)

historically influenced prices; however, recent price increases can also be linked to two more recent and related phenomena: instability and volatility in global oil markets and the role of increased demand for biofuels as alternative energy sources.

The role of global oil markets

The global price of crude oil has increased rapidly over a two year period. This has led to increased on-farm fuel costs as well as increased transportation costs for farmers. Additionally, on-farm costs have increased due the rapid increase in fertilizer prices. In conjunction with sharp increases in global crude oil prices, global natural gas prices experienced a similar sharp increase in a relatively short period of time. Since natural gas can comprise up to 90% of fertilizer production costs, the sharp increase in natural gas prices had a significant impact on fertilizer prices. Fertilizer is an important input in the production of commodities such as corn and soybeans; therefore, farm level production costs were further influenced by this increase in input prices. These increases in on-farm production costs influenced the production decisions of farmers, which in turn impacted plantings, harvest quantities, and ultimately prices.

Another key factor influencing the production and planting decisions of farmers was the increased demand for biofuels as alternative energy sources. As the price of crude oil rose, so too did the economic return associated with biofuel production, and in particular, ethanol production. Furthermore, ethanol mandates and ethanol subsidies in the United States provided farmers with an even greater incentive to transition production towards crops used in ethanol production (corn) at the expense of other crops. This led farmer to both divert corn harvests from human consumption towards ethanol production, as well as transition their fields towards greater corn production at the expense of other crops, including soybeans. This shift in crop composition and destination ultimately influenced the prices of these commodities.

These factors influenced the prices and production levels of key commodities, including corn and soybeans. Agricultural prices were driven higher as on-farm production costs increased and farmers attempted to reduce costs by substituting away from high cost inputs or away from crops with higher relative production costs. Reducing the use of fertilizer could reduce on-farm costs; however, it could also potentially result in lower harvest yields. Switching to crops with lower relative fuel and fertilizer costs or to crops with high economic returns would shift composition and number of crops planted. This would ultimately influence the supply of those commodities in the market, thereby driving prices upward. While the economic impact of these factors have yet to be discerned empirically, the fact remains that during this period of high crude oil prices, key feed ingredient prices were driven to all time record highs. The production decision of farmers to substitute towards corn production and away from high cost inputs helped fuel this increase.

Assessing the impact of recent price increases on protein production

Given this price environment, the next question to ask is: what effect has the recent price increases had on feed costs and production costs for selected animal proteins? Researchers in the US and Norway assessed the recent impact of rising feed and production costs for selected animal proteins and found that aquaculture species, and in particular salmon and shrimp, gained a cost competitiveness relative to broilers and catfish (Anderson *et al.*, 2008). This finding can be attributed to the fact that shrimp and salmon have diets that are more heavily based on marine based feed ingredients relative to catfish and broilers, which have diets that are more dominated by agricultural ingredients, including corn and soybeans. Since agriculturally based crops recently experienced sharper price increases relative to marine based ingredients, those diets were impacted more acutely relative to the more marine based diets. However, the cost competitiveness of salmon and shrimp can also be attributed to other key reasons.

Aquaculture and its relative advantages in protein production

The aquaculture industry has some production advantages relative to its agricultural counterparts. The first advantage is associated with the edible and premium meat yields of aquaculture species. For example, compared to poultry which has a final meat yield of less than 40% and a premium meat yield of approximately 15%, salmon have an edible meat yield of 60%, of which the majority of that fillet is considered premium cut (Forester, 1999). Another production advantage for aquaculture species is the feed conversion ratio (FCR) for some aquaculture species relative to land-based animals. For example, salmon and tilapia have FCRs that are lower than broilers, pork and ruminants (Table 1). This means that it takes fewer pounds of feed to produce one pound of animal growth. This is an important advantage given that feed costs compose a significant percentage of on-farm production costs (40-70%). Therefore, species that have lower feed requirements will have a competitive advantage, even if the overall prices of key feed ingredients rise.

Table 1. FCR for selected animal proteins

Salmon	~ 1.2
Tilapia	1.6 – 1.8
Broiler	2.0 – 4.0
Pork	3.4 – 3.6
Ruminants	~ 7 – 10

Source: FAO (2007), Guttormsen (2008), Pork.org (2008)

Incentives to innovate in the aquaculture industry

Following the sharp increases in fish meal and fish oil prices back in 2005, the farmed salmon industry invested in feed research and development to increase the substitution possibilities across key feed ingredient. Research focused on identifying alternative protein sources that allowed feed

manufactures to tailor diets that were both nutritionally balanced and cost competitive. In general, the identification of alternative feed ingredients expands the nutritional knowledge base for a given species. This is important for two reasons. First, the knowledge gained can be used to improve overall production of the species: improve growth rates, reduce mortality and improve the overall quality and yield of the species. These advancements are important for long-term growth and economic viability. Second, the nutritional knowledge gained through feed research is also very useful in the short-term, particularly in situations where the prices of key fed ingredients are high and/or volatile.

Capitalizing on feed Ingredient price arbitrage opportunities

Innovations that lead to increased feed knowledge and the identification of alternative feed ingredients or formulations are important because they afford producers the ability to capitalize on nutritional knowledge when the relative prices of key ingredients change. If the price of a key feed ingredient increases relative to a lower cost substitute, then producers who have the nutritional knowledge regarding potential substitution possibilities can take advantage of price differentials between ingredients by altering the feed diet in such a way that the nutritional integrity of the diet is maintained at a lower cost. Without this nutritional knowledge, producers cannot capitalize on short-term price fluctuations. This is why research that focuses on feed and nutritional development and the identification of alternative feed sources is so critical. Not only does it enhance long-term growth and economic performance, but it can also enhance short-term economic performance by allowing producers the flexibility to choose among a greater range of ingredients that have been identified through feed research. Feed research and innovation increases the portfolio of feed options available to a producer and creates feed ingredient arbitrage opportunities, allowing producers the ability to capitalize on nutritional knowledge when relative prices change. For example, Fig. 2 presents the price ratios of soybean oil/fish oil and rapeseed oil/fish oil. For the majority of the 1990s, the price ratios for both series were greater than 1, indicating that fish oil had been the cheaper ingredient relative to either soybean oil or rapeseed oil. However, since 2001 the price ratios have fallen below 1. In particular, the price ratio for soybean oil/fish oil has been below 1 since 2005, indicating that the price of soybean oil is cheaper relative to fish oil. Thus, even if the prices of both fish and soybean oil increase, in relative terms, soybean oil has a cost advantage relative to fish oil. If a producer had nutritional knowledge regarding the substitution possibilities associated with substituting soybean oil for fish oil in the diet of a given species, then he or she could take advantage of this price differential. Therefore, feed knowledge can be used to smooth input price fluctuations so that output prices or profit margins do not fluctuate widely, even if there is a significant degree of price volatility in underlying key feed ingredients.

Examples of innovation in the farmed salmon industry

The farmed salmon industry has invested in feed and nutritional research which has allowed the industry to reduce its inclusion rate for fish meal and fish oil. It has done so in part by identifying alternative feed ingredients. In 1998, the inclusion rates for fish meal and fish oil were 45% and 25%, respectively. By 2008, the inclusions rates for fish meal and fish oil were 30% and 14%, respectively, and by 2018, the industry anticipates inclusion rates of 15% and 5%, respectively for fish meal and fish oil (Skretting, 2008). The industry was able to substitute away from fish meal and fish oil by identifying alternative ingredients and expanding the variety of ingredients that could be used to formulate a diet for Atlantic salmon. This allows producers the ability to dampen price fluctuations in key feed ingredient prices by substituting towards lower cost ingredients during periods of price volatility. Further, even if real prices ultimately decline, the innovations and the knowledge gained through feed research and the cost competitiveness associated with those developments will remain.

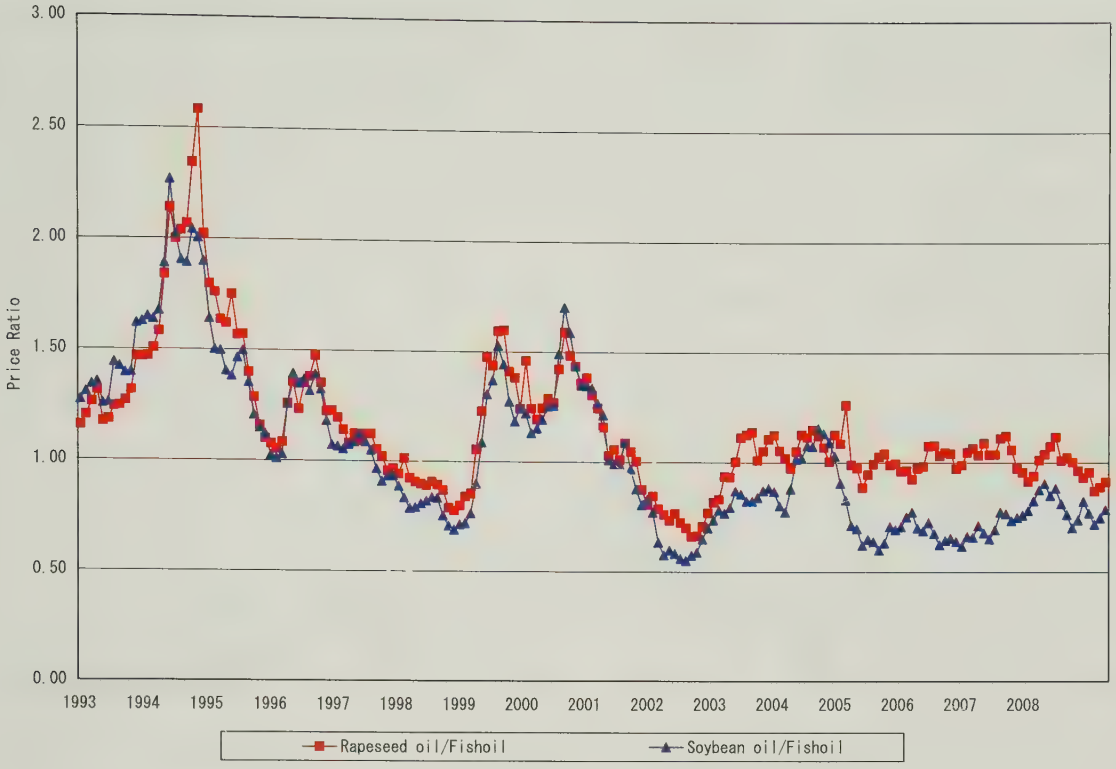

Fig. 2. Price ratios of key feed ingredients

Lessons from the U.S. poultry industry

Before the advent of the broiler industry, chicken meat was a by-product of the egg industry. Today, the U.S. broiler industry is the world's largest producer and one of the top exporters of poultry meat. The story of the U.S. poultry industry's ascendancy to the number one producer of poultry meat globally is a story of innovation in production, nutrition, processing and marketing. Fig. 3 depicts the long-run price trends for key feed ingredients (real prices) from 1914 to the present. While the long-term trend in real prices is downward, there are clear periods of volatility in key feed ingredient prices. A question to ask is, how was the U.S. poultry industry able to weather these periods of price volatility. Fig. 4 depicts the real live-weight price trend for broilers over time since 1945. As one can see from Fig. 4, the real live-weight broiler price has trended downward over time. The industry was able to weather periods of price volatility and maintain a downward trend in prices through innovation in production and husbandry practices and through substitution across key feed ingredients. Table 2 presents a historical snapshot of key production parameters for the U.S. poultry industry. Improvements have been made across all parameters over time, reflecting improvement

Table 2. Improvements in the poultry industry

	1925	1945	1965	1985	2005
FCR	4.7	4.0	2.4	2.0	1.8
Mortality	18%	10%	6%	5%	4%
Age (days)	112	84	63	49	42
Live Weight (kg)	1.0	1.4	1.6	1.9	2.4

Source: P. Aho (2004) cited by C. Lohawatanakul (2008)

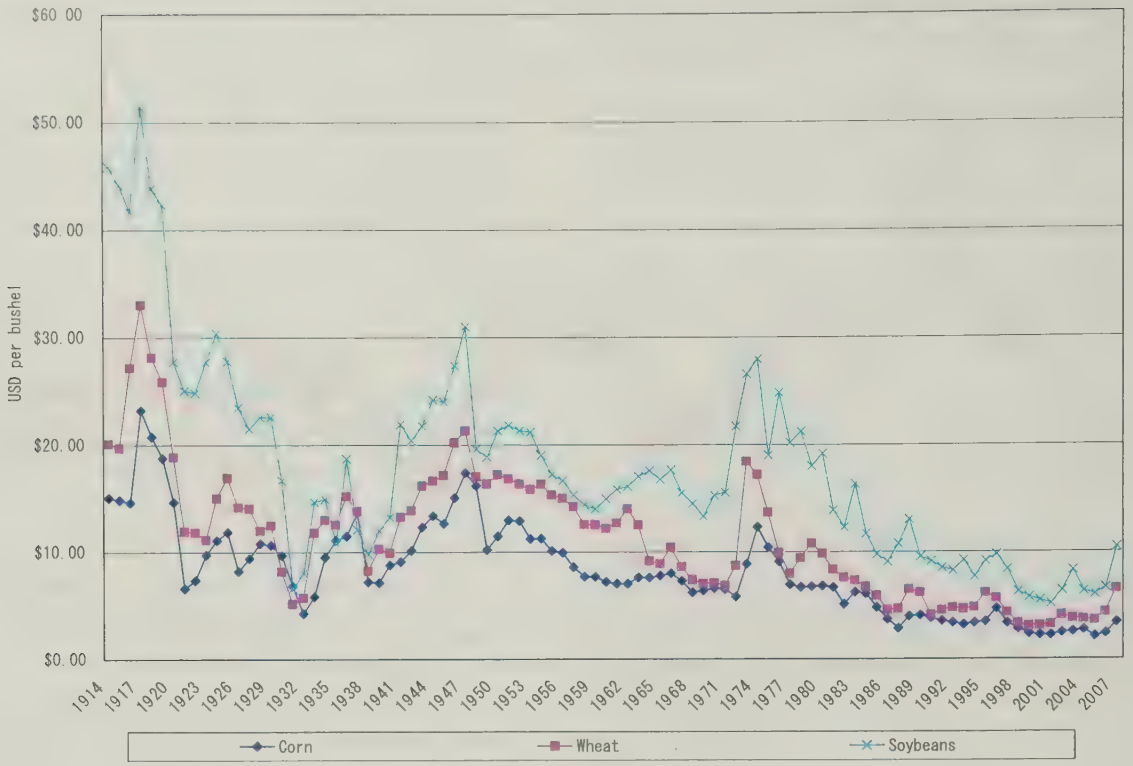

Fig. 3. Long run price trends for key feed ingredients (US dollars per bushel)

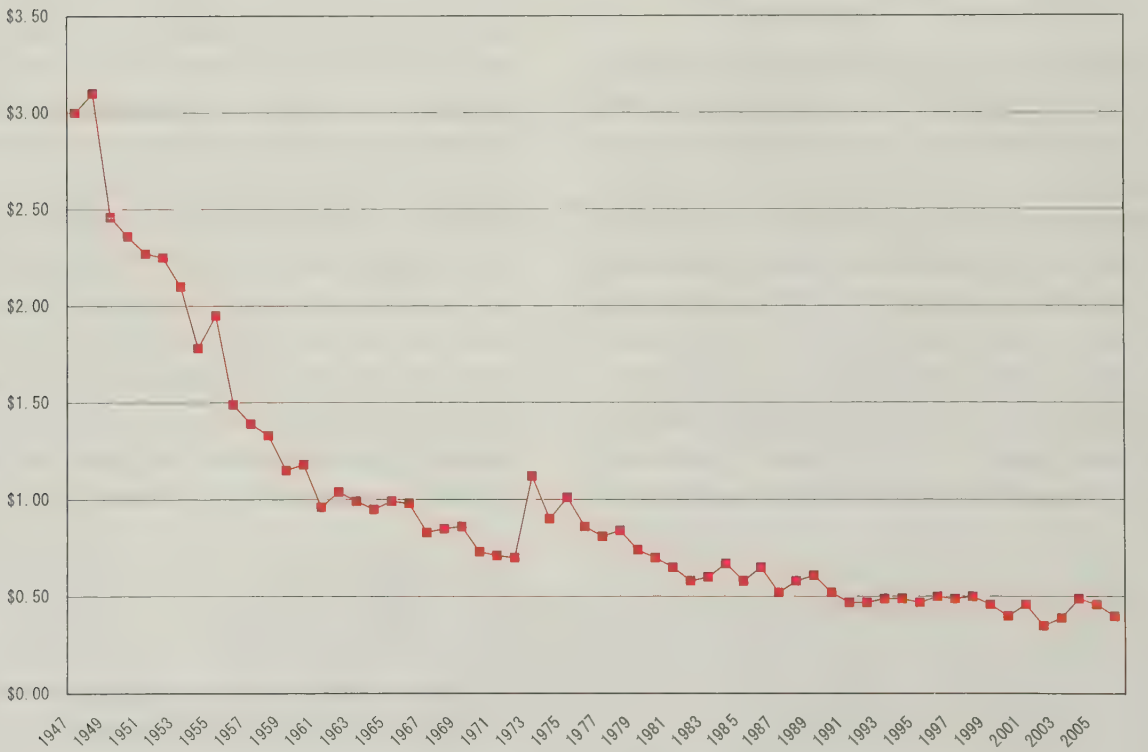

Fig. 4. Real average live weight price of broilers (US dollars per pound)

that benefited both the short-term and long-term viability of the industry. This explains in part how the industry was able to maintain declining real prices for broilers over time despite the presence of multiple periods of price volatility in key ingredients and on-farm costs.

Conclusions

A key objective of this research was to emphasize the important of feed innovations and research in identifying alternative feed sources and in improving the existing nutritional knowledge base. Such developments benefit the industry in both the long and short-term. Short-term fluctuations in key ingredient prices can be controlled at the farm level if farmers have the nutritional knowledge regarding substitution possibilities across alternative feed ingredients. The current prices of key feed ingredients have retreated from their recent highs; however volatility has always been and will continue to be present in these commodities. Therefore, nutritional knowledge and the identification of alternative feed ingredients are critical in dealing with high and volatile prices for key ingredients. Innovations allow for substitution across feed ingredients, which will help make feed costs and production costs less volatile, despite any underlying volatility in primary ingredient prices.

References

Anderson, J.L., Asche, F., and Tveteras, R., 2008: Implications of Changes in Primary Commodity Prices on the Competitiveness of Aquaculture Products. Aquavision Presentation: September 30, 2008. Stavanger, Norway.

Anderson, J.L, 2003: International Seafood Trade. Woodhead Publishing Ltd., Cambridge, England. p. 22

Forester, J. 1999: Aquaculture Chickens: Salmon- A Case Study. *World Aquaculture,* **30(3)**, 33-40.

Skretting Test Farm Tour. Personal communication. September 29, 2008.

Bibliography of Key Works

1. *Offshore Aquaculture in the United States: Economic Considerations, Implications and Opportunities.* National Oceanic and Atmospheric Administration. National Aquaculture Program. Silver Spring, MD. July 2008 (Pre-Publication Copy)
http://aquaculture.noaa.gov/news/econ.html

The report by leading fisheries and resource economists and business experts, *Offshore Aquaculture in the United States: Economic Considerations, Implications & Opportunities,* examined a range of topics on the industry's potential and found that a significant domestic offshore aquaculture industry could develop and be successful over the next 20 years with a clear regulatory framework. One chapter in particular (Chapter 4) examines the future of aquaculture feeds and feed costs by examining the role of fish meal and fish oil. The authors suggest that the limited supply of fish meal and fish oil is not likely to be a constraint on the expansion of U.S. aquaculture as alternative feed ingredients from soybeans and other plants, algaes, yeasts, fish processing waste, and other products are being developed.

2. Jackson, Andrew. 2007. Challenges and Opportunities for the Fishmeal and Fish oil Industry. Feed Technology Update. Volume 2, Issue 1. January 2007.
http://www.aquafeed.com/newsletter_pdfs/nl_000245.pdf

This paper first examines the current state of the global fish meal and fish oil industry and then examines the claim that fish meal and fish oil might limit the future growth of the aquaculture industry. The author asserts that fishmeal and fish oil will become more strategic dietary ingredients, rather than limiting the growth of the aquaculture industry.

3. Kristofersson, Dadi. and James L. Anderson. 2006. "Is There a Relationship between Fisheries and Farming? Interdependence of Fisheries, Animal Production and Aquaculture" *Marine Policy*, Vol. 30, No. 6: 721-72

These authors examine the role of fishmeal as a critically important feed ingredient for use in animal (e.g., poultry and pork) and aquaculture production (e.g., salmon, trout, and shrimp). Their research suggests that since 1998 market for fishmeal has changed. An important explanation is likely to be the increasing use of specialized feed formulations in the pork, poultry and aquaculture sectors. It is clear that growth in aquaculture production alone is insufficient to explain the structural change since growth in the use of fishmeal in aquaculture has slowed considerably despite the continued rapid growth in global aquaculture sector. The increasing relative price of fishmeal is likely to increase costs for animal producers, act as a stimulus for innovation and have considerable implications for the management of pelagic fisheries.

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Availability of Genetically Modified Feed Ingredients for Rainbow Trout *Oncorhnchus mykiss*

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Abstract : The feeding studies were conducted to assemble the information on the availability of gene-modified (GM) feed ingredient for rainbow trout, using diet containing GM defatted soybean meal (SBM) as an alternative protein source to provide and ensure good protein accessibility and product safety. The utilization of genetically modified defatted soybean meal (GM SBM) as feed by rainbow trout was investigated, in comparison with non genetically modified defatted soybean meal (non-GM SBM). The nutrient utilization showed that there was no significant difference in growth and feed performance between GM and non-GM SBM groups in 12 week feeding experiment. However, the cauliflower mosaic virus 35S promoter fragment of the GM SBM was detected in the muscle of fish receiving GM SBM diet by Nested-PCR. Additionally, the promoter fragment vanished by the 5th day after changing the diet to non-GM diet. Subsequently, the study was carried out to examine the degradation and the possible carry over of foreign DNA fragment by means of measuring it from transgenic plant and host plant contained in GM or non-GM SBM and evaluate the safety for fish. These foreign DNA fragments were not completely degraded in stomach and intestine and might be taken up into organ via the garsrointestinal (GI) tract. However, foreign DNA was not detected after the withdrawal period. Judging from these findings, the novel feed ingredients derived from GM SBM could be considered as having equivalent nutritional quality and verifying the safety as feed ingredient.

Aquaculture is increasing as an important contributor to economic development and to the global food supply. Nearly one-third of the fish consumed by humans is the product of aquaculture, and that percentage will only increase as aquaculture expands and the world's conventional fish catch from the ocean and freshwater continues to decline because of overfishing and environmental damage (FAO, 2000; OECD, 2001). Consequently, the needs for commercial feeds for intensively cultivating fish are increasing.

Most prepared fish feeds use soybean meal as a good quality plant protein (Halver and Hardy, 2002). Since genetically modified (GM) soybean meal (SBM) has been developed, it might be used as a feed ingredient for prepared feeds. Thus, the consequences of changing fish diet formulations on final product safety and quality need to be investigated. There are two important issues considered in the safety assessment of GM crops used as fish feed ingredients. First is fish safety which is assessed through feeding studies to evaluate the equivalence of nutritional performance. The second is food safety that is determined by the digestibility of the transgenic protein and its incorporation within the fish (Brown *et al.*, 2003; Sanden *et al.*, 2004).

The present article reviews information on the usefulness of GM feed ingredients for rainbow trout through formulations combining GM SBM as an alternative protein source that provides good protein availability and product safety. A series of studies

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were conducted to assess various combinations of soy protein from GM SBM and non-genetically modified defatted soybean meal (non-GM SBM) as substitutes for fishmeal. Subsequently, a study was conducted to determine the effect of GM SBM diets as a means to possibly transfer foreign DNA from the GM SBM protein to fish.

Availability of genetically modified soybean meal in rainbow trout diets

The availability of SBM as a replacement for fishmeal has been practiced for many years. Feeding studies have shown that SBM is a good protein supplement for fishmeal and can be incorporated in diets for growing rainbow trout (Cho *et al.*, 1974; Pongmaneerat and Watanabe, 1993; Tacon *et al.*, 1983). Rainbow trout were able to grow at a similar rate with a fishmeal based diet replaced with 30% defatted soybean meal (Pongmaneerat and Watanabe, 1992; Refstie *et al.*, 2000). The effects of soybean meal inclusion in diets for rainbow trout showed that no differences were observed at up to 40–50% replacement (Refstie *et al.*, 2000).

Research has been conducting showing that soybean meal produced from GM soybeans is comparable in chemical composition to conventional soybean meal (Padgette *et al.*, 1996). Other feed ingredient studies showing the nutrition equivalency of glyphosate-tolerant and conventional maize have been conducted with dairy cattle (Folmer *et al.*, 2000), sheep (Donkin *et al.*, 2000), and poultry (Brake and Vlachos, 1998). One study examined the nutrition bioequivalence of soybean meal prepared from non-GM or GM soybeans on a short-term basis in several species (Hammond *et al.*, 1996), though nutrient utilization studies in various aquatic animals are insufficient.

Utilization of genetically modified feed ingredient

The results of feeding studies, as measured by growth, feed conversion and composition, showing the nutrition equivalency of herbicide-tolerant and conventional soybean meals have been reported for catfish (Hammond *et al.*, 1996) and Atlantic salmon (Sanden *et al.*, 2004). Moreover, GM soybean meal, at the 12% inclusion level, was as safe as conventional soybean meal, at least in terms of its effect on histological parameters in the Atlantic salmon intestinal tract (Sanden *et al.*, 2005) and on health (Hemre *et al.*, 2005). GM maize has been studied in Atlantic salmon (Sanden *et al.*, 2005), poultry (Aeschbacher *et al.*, 2005; Rossi *et al.*, 2005; Tony *et al.*, 2003b), and cattle (Erickson *et al.*, 2003). The results showed that there are no existing reports where significant differences between conventional and genetically modified feeds were found.

Consequently, Chainark *et al.* (2006) investigated the utilization of genetically modified defatted soybean meal (GM SBM) as feed for rainbow trout in comparison with non-genetically modified defatted soybean meal (non-GM SBM). Both meals were included at levels of around 15 and 30% in four diets (42% protein). The diets were fed to juvenile fish (48.3 g on average weight) for 12 weeks. Table 1 shows the results of the feeding experiment. There was no significant difference in growth and feed performance between the GM and non-GM SBM groups at either inclusion level at the end of 12th week. The cauliflower mosaic virus 35S promoter fragment (220 bp) of the GM SBM was detected in the muscle of fish receiving both levels of GM SBM diet by Nested-PCR, with the frequency of detection being greater at the higher inclusion level (Table 2 and Fig.1). Additionally, the promoter fragment was not detected by the 5th day after changing the diet to a non-GM ration. Conversely, the promoter fragment was not detected from fish fed with non-GM SBM formulations. The results demonstrated that the availability of protein in GM SBM was similar to that of non-GM SBM, and the promoter fragments which were found in the muscle of fish were not detectable after changing the diet to non-GM diet, verifying the availability of the GM SBM in rainbow trout feed.

Investigations of ingested foreign DNA in rainbow trout

A number of studies have now been conducted in which foreign DNA derived from GM feed ingredients has not been detected in food products derived from livestock receiving GM feed. Studies have been conducted on poultry (Ash *et al.*, 2000),

Table 1. Growth and feed performance in rainbow trout fed diets graded levels of non-GM and GM SBM for 12 weeks.

Diet	Body weight (g)		SGR*[1]	FGR*[2]	Protein Retention(%) *[3]	PER*[4]
	Initial	Final				
non-GM SBM 15%	48.2±0.5	143.6±0.3	1.30±0.01	0.99±0.01	39.5±0.1	2.37±0.01
non-GM SBM 30%	47.8±0.2	140.8±0.6	1.28±0.01	1.03±0.01	38.3±0.4	2.28±0.03
GM SBM 16%	48.7±0.8	143.5±1.3	1.29±0.01	0.98±0.01	39.8±0.1	2.39±0.01
GM SBM 31%	48.5±1.0	141.6±0.1	1.28±0.02	1.01±0.02	39.0±0.7	2.32±0.05

The values were not significantly different ($P<0.05$)

*[1] SGR (Specific Growth Rate) = {(Ln Final body weight (g) - Ln Initial body weight (g)) / Experimental period (days)} × 100

*[2] FGR (Feed Gain Ratio) = Feed consumption (g) / Weight gain (g)

*[3] Protein retention = {(Final body weight (g) × Protein (%) - Initial body weight (g) × protein (%)) / Feed consumption (g) × Protein (%)} × 100

*[4] PER (Protein Efficiency Ratio) = Weight gain (g) / Protein consumption (g)

Table 2. Detectable CaMV 35S promoter fragment of recombinant DNA (220 bp) by Nested-PCR in muscles of rainbow trout fed diets graded levels of non-GM and GM SBM for 15 weeks and after withdrawing GM SBM.

Sampling day (week) /Fish number sampled	Fed with Non-GM and GM SBM diets		Fed with Non-GM SBM diet		
	84 (12) / 20	105(15) / 5	2 / 5	5 / 5	8 / 5
non-GM SBM 15%	0	0	0	0	0
non-GM SBM 30%	0	0	0	0	0
GM SBM 16%	1	1	0	0	0
GM SBM 31%	4	2	1	0	0

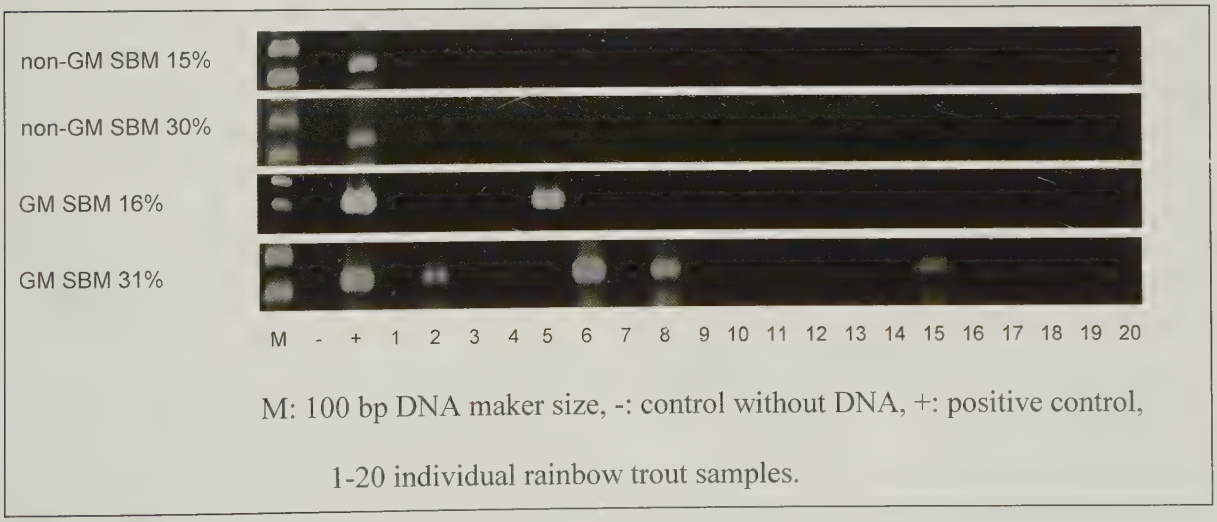
non-GM SBM 15%
non-GM SBM 30%
GM SBM 16%
GM SBM 31%
M - + 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
M: 100 bp DNA maker size, -: control without DNA, +: positive control,
1-20 individual rainbow trout samples.

Fig. 1. Detectable CaMV 35S promoter fragment of recombinant DNA (220 bp in length) by Nested-PCR in muscles of rainbow trout fed graded levels of non-GM and GM SBM diets at the end of 12th week.

swine (Weber and Richert, 2001), and dairy cows (Phipps *et al.*, 2002). Interestingly, small fragments of plant DNA have been detected in various animal gastrointestinal tracts, e.g. the intestines of fish (Sanden *et al.*, 2004), in some animal tissues from fish (Nielsen *et al.*, 2006; Nielsen *et al.*, 2005), swine (Klotz *et al.*, 2002; Reuter and Aulrich., 2003) chickens (Chambers *et al.*, 2002; Einspanier *et al.*, 2001), cattle (Einspanier *et al.*, 2001), and in bovine saliva and rumen fluid (Duggan *et al.*, 2000). However, until now, few studies have been conducted on aquatic animals.

Chainark *et al.* (2008) reported degradation and the possible carryover of foreign DNA fragments by means of measuring it from transgenic plants and host plants contained in GM or non-GM SBM formulated diets and evaluated the safety for fish. For that study, the experimental diets were formulated with GM and non-GM SBM at a level of 30%. The two experimental diets, also included fishmeal to achieve a 42% protein level. Initially, 240 rainbow trout averaging 50.5 g were fed the non-GM SBM diet for two weeks. Thereafter, the fish were divided into two groups, each of which was fed one of the experimental for an additional two weeks, then sampled. Fish fed the GM diet were then given the non-GM diet and sampled 1st, 3rd, 5th and 7th day after being placed on that diet. The degradation of digesta in the gastrointestinal (GI) tract (stomach, anterior and posterior intestine) and possible transfer of foreign DNA into various organs (blood, head kidney, spleen, liver, muscle and brain) were examined. Foreign DNA fragments, such as CaMV 35S promoter (220 bp) and *Glycine max* chloroplast (257 bp) were traced by Nested-PCR and located by *in situ* hybridization (ISH). The chloroplast DNA fragment was amplified in non-GM and GM SBM diets, but promoter DNA fragment was detected only in the GM SBM diet, indicating that cross contamination of the non-GM SBM could be ruled out. The promoter DNA fragment was detected in the contents of the GI tracts of fish fed the GM SBM diet, but chloroplast DNA fragment was amplified from fish fed non-GM or GM SBM diet. Moreover, promoter fragment was not detected on the 3rd day after changing the diet (Table 3). The promoter DNA fragment was detected in the blood, head kidney and muscle of fish fed the GM SBM diet, but not in the spleen, liver or brain. Promoter fragment was not detected on the 5th day after the switch over (Table 4). No promoter DNA fragment was detected in blood or other tissues of fish fed the non-GM SBM diet. Chloroplast DNA fragment was detected in blood and some tissues of fish fed either the non-GM or GM SBM diet. ISH analysis confirmed that the promoter and chloroplast DNA were found in tissues. These results suggested that foreign DNA fragments were not completely degraded in the stomach and intestine and might be taken up into organs via the GI tract. However, foreign DNA was not detected after the withdrawal period. Thus, the uptake of DNA from GM SBM might be regarded as safe as non-GM SBM.

These series of studies have demonstrated that an appropriate combination of GM and non-GM SBM could be a good protein source without leading to a significant loss in growth performance. In addition, the DNA from GM SBM found in fish fed a diet

Table 3. Detection of CaMV 35S promoter DNA fragment (220 bp) in contents of GI tract from fish (n=20) fed GM SBM diet at the end of the 2nd week and after changing the diet to non-GM SBM diet by Nested-PCR.

Fed with	GM SBM diet	non-GM SBM diet			
Sampling day	15	1	3	5	7
Stomach	8	2	0	0	0
Anterior intestine	5	3	0	0	0
Posterior intestine	2	5	0	0	0

Table 4. Detection of CaMV 35S promoter fragment (220 bp) in leukocyte and tissues of fish (n=20) fed GM SBM diet at the end of the 2nd week and after changing the diet to non-GM SBM diet by Nested-PCR.

Fed with	GM SBM diet	non-GM SBM diet			
Sampling day	15	1	3	5	7
Leukocyte	2	3	1	0	0
Head kidney	1	1	2	0	0
Spleen	0	0	0	0	0
Liver	0	0	0	0	0
Muscle	1	1	0	0	0
Brain	0	0	0	0	0

containing it vanished after a certain period of time. Furthermore, gene expression initiated by the promoter derived from GM SBM was not observed in fish cells. Judging from these findings, the novel feed ingredients derived from GM SBM could be considered as having equivalent nutritional quality and verify their safety. Therefore, it is considered that the GM SBM might be potentially useful in developing diets for rainbow trout and the other fish species though the required withdrawal period should be determined on a species-by-species basis.

References

Aeschbacher, K., Messikommer, R., Meile, L., and Wenk, C., 2005: Bt176 corn in poultry nutrition: Physiological characteristic and fate of recombinant plant DNA in chicken. *Poult. Sci.*, **84**, 385-394.

Ash, J.A., Scheideler, S.E., and Novak, C.L., 2000: The fate of genetically modified protein from Roundup Ready soybeans in the laying hen. *Poult. Sci.*, **79 (Suppl. 1)**, 26 (Abstr.).

Brake, J., and Vlachos, D., 1998: Evaluation of transgenic event 176 Bt corn in broiler chickens. *Poult. Sci.*, **77**, 648-653.

Brown, P.B., Wilson, K.A., Jonker, Y., and Nickson, T.E., 2003: Glyphosate tolerant canola meal is equivalent to the parental line in diets fed to rainbow trout. *J. Agric. Food Chem.*, **51**, 4268-4272.

Chainark, P., Satoh, S., Hino, T., Kiron, V., Hirono, I., and Aoki, T., 2006: Availability of genetically modified soybean meal in rainbow trout (*Oncorhynchus mykiss*) diets. *Fish. Sci.*, **72**, 1072-1078

Chainark, P., Satoh, S., V., Hirono, I., Aoki, T., and Endo, M., 2008: Availability of genetically modified feed ingredient II – Investigations of ingested foreign DNA in rainbow trout, *Oncorhynchus mykiss*. *Fish. Sci.*, **74**, 380-390

Chambers, P.A., Duggan, P.S., Heritage, J., and Forbes, J.M. 2002: The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. J. Antimicrob. *Chemother.*, **49**, 161-168.

Cho, C.Y., Bayley, H.S., and Slinger, S.J., 1974: Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout. *J. Fish Res. Bd. Canada*, 1523-1528.

Donkin, S., Velez, J.C., Stanisiewski, E.P., and Hartnell, G.F., 2000: Effect of feeding Roundup Ready corn silage and grain on feed intake, milk production and milk consumption in lactating dairy cattle. *J. Dairy Sci.*, **83 (Suppl.1)**, 273 (abstr.).

Duggan, P.S., Chamber, P.A., Heritage, J., and Forbes, J. M., 2000: Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiology Lett.*, 191, pp.

Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwagele, F., Jahreis, G., and Flachowsky, G., 2001: The fate of forage plant DNA in farm animals: A collaborative case-study investigating cattle and chicken fed recombinant plant material. *Eur. Food Res. Technol.*, **212**, 129-134.

Erickson, G.E., Aarts, H., Buhk, H.J., Corthier, G.,

Flint, H.J., Hammes, W., Jacobsen, B., Midtvedt, T., Vossen, J.V., Wright, A.V., Wackernagel, W., and Wilcks, A., 2003: Effect of feeding glyphosate-tolerant (Roundup-ready events G21 or NK603) corn compared with reference hybrids on feedlot steer performance and carcass characteristics. *J. Anim. Sci.*, **81**, 2600-2608.

FAO, 2000: Agriculture: Towards 2015/30:Technical interim report, Rome.

Folmer, J.D., Grant, R.J., Milton, C.T., and Beck, J.F., 2000: Effect of Bt corn silage on short-term lactational performance and ruminal fermentation in dairy cows. *J. Dairy Sci.*, **83**, 1182 (Abstr.). C. T.

Halver, J.E., and Hardy, R.W., 2002: Fish nutrition 3rd edtion. Academic Press, California, 824pp.

Hammond, B., Vicini, J.L., Hartnell, G.F., Naylor, M.W., Knight, C.D., Robinson, E.H., Fuchs, R.L., and Padgette, S.R., 1996: The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *J. Nutr.*, **126**, 717-727.

Hemre, G.I., Sanden, M., Bakke-McKellep, M.A., Sagstad, A., and Krogdahl, Å., 2005: Growth, feed utilization and health of Atlantic salmon (*Salmo salar* L.) fed genetically modified compared to non-modified commercial hybrid soybeans. *Aquacult. Nutr.*, **11**, 157-167.

Klotz, A., Mayer J, and Einspanier, R., 2002: Degradation and possible carry over of feed DNA monitored in pigs and poultry. *Eur. Food Res. Technol.*, **214**, 271-275.

Nielsen, C., Berdal, K.G., and Holst-Jensen, A., 2006: Persistence and distribution of intravenously injected DNA in blood and organs of Atlantic salmon (*Salmo salar* L.). *Eur. Food Res. Technol.*, **222**. 258-265.

Nielsen, C., Berdal, K.G., Bakke-McKellep, A.M., and Holst-Jensen, A., 2005: Dietary DNA in blood and organs of Atlantic salmon (*Salmo salar* L.). *Eur. Food Res. Technol.*, **221**. 1-8.

OECD. 2001. OECD environmental outlook, Paris.

Padgette, S.R., Taylor, N.B., Nida, D.L., Bailey, M.R., Macdonald, J., Holden, L.R., and Fuchs, R.L., 1996: The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J. Nutr.*, **126**, 702-716.

Phipps, R.H., Beever, D.E., and Humphries, D.E., 2002: Detection of transgenic DNA in milk from cows receiving herbicide tolerant (CP4EPSPS) soybean meal. *Livest. Prod. Sci.*, **73**, 269-273.

Pongmaneerat, J., and Watanabe, T., 1992: Utilization of soybean meal as a protein source in diets for rainbow trout. Nippon Suisan Gakkaishi, **58**, 1983-1990.

Pongmaneerat, J., and Watanabe, T., 1993: Nutrition evaluation of soybean meal for rainbow trout and carp. Nippon Suisan Gakkaishi, **59**, 157-163.

Refstie, S., Korsøen, Ø.J., Storebakken, T. , Baeverfjord, G., Lein, I., and Roem, A.J., 2000: Differing nutritional responses to dietary defatted soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.). *Aquculture*, **190**. 49-63.

Reuter, T., and Aulrich, K., 2003: Investigations on genetically modified maize (Bt-maize) in pig nutrition: Fate of feed-ingested foreign DNA in pig bodies. *Eur. Food Res. Technol.*, **216**, 185-192.

Rossi, F., Morlacchini, M., Fusconi, G., Pietri, A., Mazza, R., and Piva, G., 2005: Effect of bt corn on broiler growth performance and fate of feed-derived DNA in the digestive tract. *Poult. Sci.*, **84**, 1022-1030.

Sanden, M., Berntssen, M.H.G., Krogdahl, Å., Hemre, G.I., and Bakke-McKellep, A.M., 2005, An examination of the intestinal tract of Atlantic salmon, *Salmo salar* L., parr fed different varietes of soy and maize. *J Fish Dis.*, **28**, 317-330.

Sanden, M., Bruce, I.J., Rahman, M.A., and Hemre, G., 2004: The fate of transgenic sequences present in genetically modified plant products in fish feed, investigating the survival of GM soybean DNA fragments during feeding trials in Atlantic salmon, *Salmo salar* L. *Aquaculture*, **237**, 391-405.

Tacon, A.G.J., Haaster, P., Featherstone, P.B., Kerr, K., and Jackson, A.J., 1983: Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Nippon Suisan Gakkaishi, **49**, 1437-1443.

Tony, M.A., Butschke, A., Zagon, J., Broll, H., Schauzu, M., Awadalla, S.A., Hafez, H.M., and

Flachowsky, G., 2003a: Incidence of genetically modified soybean and maize as animal feed in Egypt. *J. Anim. Feed Sci.*, **12**, 333-347.

Tony, M., Butschke, A., Broll, H., Grohmann, L., Zagon, J., Halle, I., Danicke, S., Schauzu, M., Hafez, H.M., and Flachowsky, G., 2003b: Safety assessment of Bt 176 maize in broiler nutrition: Degradation of maize-DNA and its metabolic fate. *Arch. Tierernahr.*, **57**, 235-252.

Weber, T.E., and Richert, B.T., 2001: Grower-finisher growth performance and carcass characteristics including attempts to detect transgenic plant DNA and protein in muscle from pigs fed genetically modified Bt corn. *J. Anim. Sci.*, **79 (Suppl. 2)**, 67 (Abstr.).

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Quantification of Waste Feed and Fish Feces using Stable Carbon and Nitrogen Isotopes

Hisashi YOKOYAMA *, Yuka ISHIHI *, Katsuyuki ABO *, and Toshinori TAKASHI *

Abstract : For improving the economic and environmental sustainability of fish farming, it is necessary to optimize feeding regimes and to decrease waste feed. We developed a method to quantify waste feed and fecal matter in sediment trap materials and sediments beside and beneath fish cages using stable carbon and nitrogen isotope ratios. The contribution ratio of three sources (waste feed, fecal matter and natural organic matter) in sedimentary organic matter in and around a red sea bream (*Pagrus major*) farm in Gokasho Bay, central Japan, was estimated using an isotopic mixing model. The result showed that waste feed (29% in the bulk organic matter) exceeded fecal matter (12%) in the fish farm area, suggesting the overfeeding in this farm. Then we monitored the growth and mortality of red sea bream, C and N fluxes and sediment chemistry at two commercial fish cages; cage 1 (conventional satiation feeding) and cage 2 (restricted feeding: same feeding frequency but 18% reduction in the feed amount) for 276 days. The restricted feeding achieved normal growth, increased feed conversion efficiency and reduced mortality of red sea bream and reduced contents of waste feed-derived organic matter in the sediment. Determining the relative amount of waste feed and fecal matter in settling and sedimentary organic matter is effective for the evaluation of the optimum ration level from the viewpoint of the minimization of waste feed.

Key words : fish farm, stable isotope, waste feed, fecal matter, feeding

Intensive fish farming in coastal waters generates large amounts of particulate organic wastes in the form of waste feed (WF) and fecal matter (FM). The particulate organic wastes settle onto the seabed and produce enriched sediments, which can result in the deoxygenation of the bottom water, the production of reduced compounds such as ammonium and sulfides and changes in the structure of benthic communities (reviewed by Pearson and Black, 2001). Such environmental deterioration often produces negative consequences for the management of the farm. The practical way to implement environmental management of fish farms is to reduce organic matter (OM) loads by selecting 'low-pollution' feed pellets such as extruded pellets (EP), which have high stability and appropriate buoyancy in water (Beveridge, 1984), instead of trash fish and moist pellets. The feeding regime is also an important husbandry factor in efforts to reduce WF (Wang *et al.*, 2007). Overfeeding leads to a loss of feed, pollutes the environment and leads to a decrease in feed conversion efficiency, while underfeeding results in increased interfish competition for feed and reduced growth and decreased feed conversion efficiency (Talbot *et al.*, 1999). In this regard, it is necessary to determine the optimum ration level, which may be variable depending on the culture species, its body size and the environmental temperature (Azevedo *et al.*, 1998). Information on the optimum ration level is also effective for the farm management, because the feed represents a major cost in fish culture. From these viewpoints, Yokoyama *et al.* (2006) developed

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a method to quantify waste feed and fecal matter in the sediment using stable carbon and nitrogen isotope ratios, and found that WF (29% in the bulk OM) exceeded FM (12%) in a fish farm in Gokasho Bay, central Japan. Based on this finding, we informed fish farmers of the cooperative in Gokasho Bay the possibility of overfeeding and proposed an investigation to the farmers to culture red sea bream (*Pagrus major*) under the conventional satiation feeding regime and a restricted feeding regime in the two neighboring fish cages in order to monitor and compare the growth and mortality of fish and environmental impacts over a period of 276 days (Yokoyama *et al.*, 2009). In this review, these results are summarized.

Method to quantify fish farm wastes in settling and sedimentary organic matter

In general, settling and sedimentary organic matter (OM) beside and beneath fish cages includes three main organic matter source, i.e., WF, FM, and natural settling or sedimentary OM derived from natural sources. Yokoyama *et al.* (2006) determined the average $\delta^{13}C$ and $\delta^{15}N$ values of feed supplied to the fish cages (as the WF value), feces excreted from the red sea bream, and natural settling or sedimentary OM as follows (Fig. 1).

WF: In the fish farm in Gokasho Bay, moist pellets (MP) and extruded pellets (EP) were used to culture red sea bream. The mean $\delta^{13}C$ and $\delta^{15}N$ values for moist pellets were −20.2‰ and 10.2‰, respectively, while those for extruded pellets (EP) were −20.3‰ and 9.1‰, respectively. Of feed supplied to the fish cages in 2002, MP and EP comprised 59% and 41% by dry weight, respectively. The overall mean values for feed supplied to the fish cages (= waste feed) were estimated as $\delta^{13}C = -20.2 \times 0.59 + (-20.3 \times 0.41) = -20.2$‰, and $\delta^{15}N = 10.2 \times 0.59 + 9.1 \times 0.41 = 9.7$‰, respectively.

FM: The mean $\delta^{13}C$ and $\delta^{15}N$ for feces excreted from the red sea bream fed on MP were −24.7‰ and 5.6‰, which were 4.4‰ reduced in ^{13}C and 3.5‰ reduced in ^{15}N relative to the feed, respectively. The mean $\delta^{13}C$ and $\delta^{15}N$ values for feces excreted from the red sea bream fed on the dry pellets were −23.7‰ and 7.2‰, which were 3.5‰ reduced in ^{13}C and 3.3‰ reduced in ^{15}N relative to the feed, respectively. Assuming that the ^{13}C and ^{15}N shifts which were found between feed and feces in the laboratory experiment are applicable to the fish population in the fish farm in Gokasho Bay, mean isotopic compositions for feces that were loaded to the environment are estimated as $\delta^{13}C = (-20.2 - 4.4) \times 0.59 + (-20.3 - 3.5) \times 0.41 = -24.3$‰, and $\delta^{15}N = (10.2 - 3.5) \times 0.59 + (9.1 - 3.3) \times 0.41 = 6.3$‰, respectively (Fig. 1).

Natural OM: Isotopic compositions of natural settling and sedimentary OM were determined by samples collected from reference stations. The $\delta^{13}C$ and $\delta^{15}N$ values of natural settling OM were −19.6‰ and 7.5‰, respectively, while those of natural sedimentary OM were −19.9‰ and 5.5‰ (Fig. 1).

The $\delta^{13}C$ and $\delta^{15}N$ values of sedimentary OM (SOM) at the 41 stations in and around the fish farm in Gokasho Bay are shown in Fig. 1. In this dual isotopic plot, most points of SOM are located in the area of a triangle which are formed by the isotopic compositions of WF, FM and SOM reference. This enables the calculation of the ratios of WF and FM in SOM based on the simple linear mixing model (Phillips, 2001). The ratios of WF and FM in sediment trap samples can be also determined based on the isotopic compositions of WF, FM and natural settling OM.

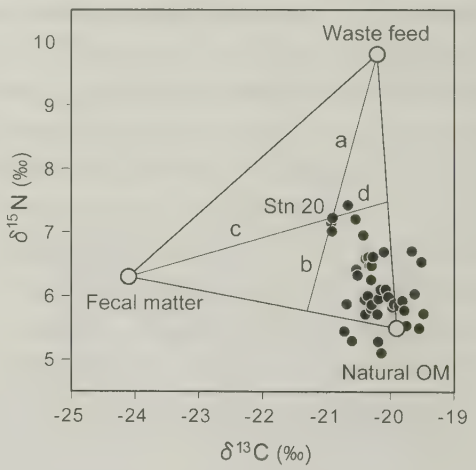

Fig. 1. Dual isotope plot for sedimentary organic matter in the fish farm (●) and the organic matter sources (○), which include feed, fish feces and natural sedimentary organic matter. For example, the ratios of waste feed and fecal matter in sedimentary organic matter at Stn 20 are calculated by {b/(a + b)} × 100%, and {d/(c + d)} × 100%, respectively (adapted from Yokoyama *et al.*, 2006).

Ratio of waste feed and fecal matter in sedimentary organic matter

Fig. 2 shows contours of the ratio of waste feed and fecal matter in SOM in and around the fish farm in Gokasho Bay. The WF ratio in the fish-farm area (within 30 m from the edge of cages) ranged from 10.3 to 43.2% (mean = 28.8%), whereas at most stations outside the fish-farm area the ratio was <10%. In the central part of the northern and southern cage rows, conspicuous high WF ratios >30% were found. The FM ratio in the fish-farm area ranged from 2.6 to 20.8% (mean = 11.9%), whereas at most stations outside the area the ratio was <10%. In the central part of the northern cage rows, high FM ratios of >20% were found. On average, the WF ratio was 2.4-fold larger than the FM ratio.

Effects of restricted feeding on fish culture and environments

The excess of WF over FM suggests the overfeeding in the fish farm in Gokasho Bay. We informed the fish farmers the possibility of overfeeding and proposed an investigation to culture red sea bream under two ration levels; cage 1 (conventional satiation feeding, which was determined by a feed table that was provided by the manufacturer and by the fish farmers' experience) and cage 2 (restricted feeding: same feeding frequency but 18% reduction in the feed amount). Then, the experimental aquaculture was conducted in these 2 cages (initial stocking density = 11000 fish in each cage) from August 23, 2006 to May 26, 2007 (276 days) and we monitored the growth and mortality of fish, carbon and nitrogen fluxes immediately beside the cages and the sediment chemistry below the cages (Yokoyama *et al.*, 2009).

There was no significant difference in fish weight in each month between the 2 cages (two sample *t*-test, all $p > 0.19$) (Fig. 3a). As shown in the standard deviations of the fish weight in the 2 cages (Fig. 3a), there was no difference in the variability of the body size between them. The similar growth rate of fish in the 2 cages (i.e., 0.43% in cage 1 vs. 0.39% in cage 2) in spite of the difference in the total amount of feed resulted from a difference in feed conversion efficiency, 0.54 in cage 1 and 0.62 in cage 2.

Dead fish were found almost every day from August through October, however, they were rarely found after the beginning of November, when the water temperature fell below 21℃ and the ration decreased under 2.2%. Cumulative number of dead

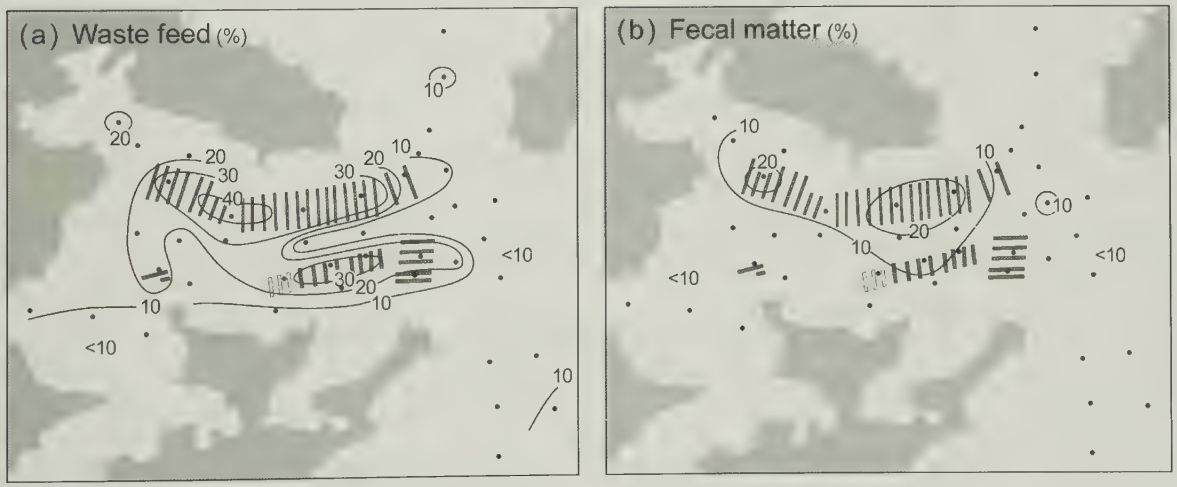

Fig. 2. Aquaculture wastes in the sediment. Isopleths of (a) waste feed ratio and (b) fecal matter ratio in sedimentary organic matter in and around a fish farm in Gokasho Bay (after Yokoyama *et al.*, 2006).

fish in cage 1 was 462, which was >2-times higher than in cage 2 (205) (Fig. 3b).

The sediment trap survey showed that the C and N fluxes beside the fish cages were several times higher than those at the control site and that the differences between the mean fluxes at the fish cages and the background ones were 1.1g C m^{-2} d^{-1} and 0.11g N m^{-2} d^{-1}, respectively. However, there were no significant differences in the C and N fluxes between the 2 fish farm stations, probably due to the close vicinity of the 2 stations (distance = 13 m). The large differences in the isotopic composition between WF, FM and natural settling OM and the intermediate isotopic values of the fish-farm settling OM between these OM sources enabled the successful calculation of the contribution rates of WF and FM. As a result, we found that (1) aquaculture-derived OM (AOM), composed of WF and FM, accounted for most of the sedimentation at the fish-farm stations, excluding the period from January to April, when natural OM often accounted for half of the bulk OM, (2) there was a difference in the seasonal trend between the WF and FM fluxes, and (3) there were no differences in the percentage compositions of WF and FM between the two fish-farm stations. Although we could not find the differences in the sedimentation rate and percentage compositions of WF and FM between the 2 fish-farm stations, there was a large variability in the relative amount of WF and FM (the WF:FM ratio, Fig. 4) both in the 2 feeding regimes. For instance, during November and December the amount of WF was scarce despite the large input of feed (low WF:FM ratios), whereas relatively large WF fluxes were found after January although there was only a small input of feed (high WF:FM ratios). This finding suggests that the WF fluxes depend not simply on the feed input but more strongly on the feed intake and digestibility of fish and that there is still scope for improvement of the feeding regime even in cage 2. The quantification of WF and FM in settling OM using the stable isotope ratios and the WF:FM ratio will serve for assessing the optimum ration size.

The stable isotope ratios were used to calculate the relative contributions of WF, FM and the natural source to the bulk sedimentary OM. The result

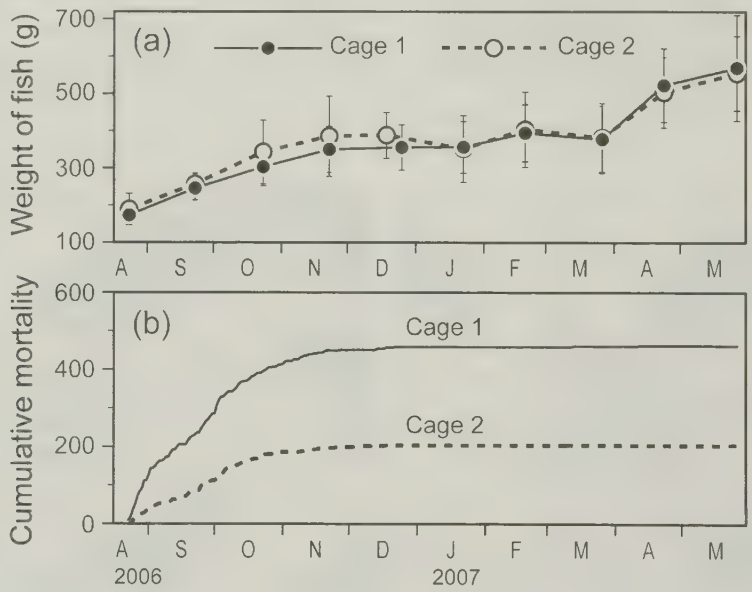

Fig. 3. Experimental aquaculture of red sea bream. (a) Growth of red sea bream and (b) cumulative number of dead fish in cage 1 (satiation feeding) and cage 2 (restricted feeding) (after Yokoyama *et al.*, 2009).

showed that on average WF contributed to 17%, FM contributed to 30% and the other source contributed to 53% of the bulk OM. Such values were distinct from those of the trap material (WF 43%, FM 43% and the natural source 14%), showing reduced contribution ratios in AOM, especially in WF in the sedimentary OM. The observed difference between them is probably due to highly labile compounds in AOM (Pearson and Black, 2001). AOM also could be incorporated into the food web through the digestion by macrobenthic animals in Gokasho Bay (Yokoyama and Ishihi, 2007) resulting in the decrease in the contribution ratios of AOM to the bulk OM.

WF-derived nitrogen contents in the sediment beneath cages 1 and 2 were calculated based on the total N contents in the sediment and the percentage compositions of WF and FM. The WF-derived N contents below cage 2 were significantly less than those below cage 1 (Fig. 5), whereas there were no significant differences in the FM contents between the two cages. This finding suggests that the effect of restricted feeding appeared in the sediment as a decrease of WF-derived OM.

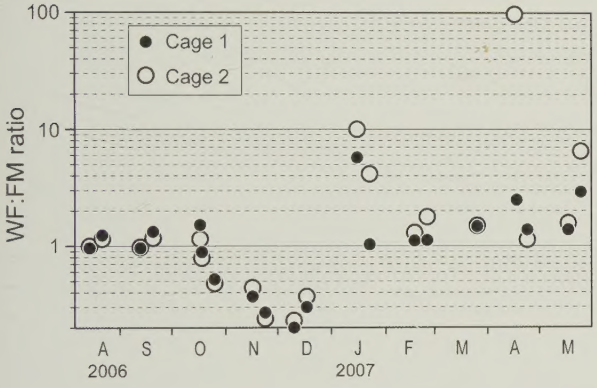

Fig. 4. Waste feed:fecal matter ratio in settling organic matter beside cage 1 (satiation feeding) and cage 2 (restricted feeding). (after Yokoyama *et al.*, 2009).

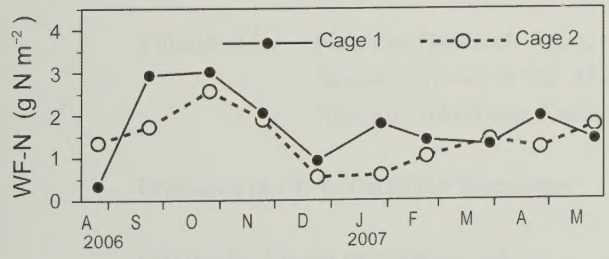

Fig. 5. Waste feed-derived nitrogen contents in the sediment below cage 1 (satiation feeding) and cage 2 (restricted feeding) (after Yokoyama *et al.*, 2009).

In conclusion, the restricted feeding, in which the amount of feed was reduced by 18% relative to the conventional satiation feeding, achieved normal growth, increased feed conversion efficiency and reduced mortality of red sea bream and reduced contents of waste feed-derived organic matter in the sediment. The stable isotope analysis was shown to be a useful tool to quantify the amount of WF- and FM-derived C and N in the sediment trap material and the sediments. Determining the relative amount of WF and FM is effective when evaluating the optimum feeding regime from the viewpoint of the minimization of WF. The present study suggests that the conventional satiation feeding for the red sea bream aquaculture is overfeeding and that the optimum ration size should be examined further.

References

Azevedo, P. A., Cho, C. Y., Leeson, S., and Bureau, D. P., 1998: Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquat. Living Resour.*, **11**, 227–238.

Beveridge, M. C. M., 1984: Cage and Pen Fish Farming. Carrying Capacity Models and Environmental Impact. *FAO Fish. Tech. Pap.* **255**, FAO, Rome, 131pp.

Pearson, T. H., and Black, K. D., 2001: The environmental impacts of marine fish cage culture. *In* K. D. Black (ed.), Environmental Impacts of Aquaculture, Sheffield Academic Press, Sheffield, p. 1–31.

Phillips, D. L., 2001: Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia*, **127**, 166–170.

Talbot, C., Corneillie, S., and Korsøen, Ø., 1999: Pattern of feed intake in four species of fish under commercial farming conditions: implications for feeding management. *Aquacult.*

Res., **30**, 509–518.

Wang, Y., Kong, L. J., Li, K., and Bureau, D. P., 2007: Effects of feeding frequency and ration level on growth, feed utilization and nitrogen waste output of cuneate drum (*Nibea miichthioides*) reared in net pens. *Aquaculture*, **271**, 350–356.

Yokoyama, H., Abo, K., and Ishihi, Y., 2006: Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. *Aquaculture*, **254**, 411–425.

Yokoyama, H., and Ishihi, Y., 2007: Variation in food sources of the macrobenthos along a land-sea transect : a stable isotope study. *Mar. Ecol. Prog. Ser.*, **346**, 127–141.

Yokoyama, H., Takashi, T., Ishihi, Y., and Abo K., 2009: Effects of restricted feeding on growth of red sea bream and sedimentation of aquaculture wastes. *Aquaculture*, **286**, 80–88.

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